SOIL QUALITY GUIDELINES FOR BARITE: ENVIRONMENTAL HEALTH AND HUMAN HEALTH

Alberta
Environment

Pub. No: T/738
ISBN No. 0-7785-3163-5 (Printed Edition)
ISBN No. 0-7785-3164-3 (On-line Edition)
Web Site: http://www.gov.ab.ca/env/info/infocentre/publist.cfm

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1. INTRODUCTION

The following report compiles and summarizes applicable background information and develops soil quality guidelines for barite (barium sulphate). Typically, guidelines are developed for trace elements, rather than for inorganic compounds of the elements. However, a specific guideline for barite is justified and required on account of (i) the very low solubility and availability of barite in comparison with the soluble barium compounds that were used to develop the CCME (1999) barium soil quality guideline, and (ii) the ubiquitous presence of barite in the soil of former oil and gas drilling sites owing to its use as a major component of oilfield drilling muds.

The barite soil quality guidelines developed herein follow Canadian Council of Ministers of the Environment (CCME 1996, 1999, 2000) protocols wherever applicable and appropriate, and only deviate from these protocols where dictated by particular issues relevant to barite.

2. BACKGROUND INFORMATION

Barite ($BaSO_4$; CAS No. 7727-43-7) is a mineral in the anhydrous sulphate series and is one of the most common naturally occurring forms of barium United States Environmental Protection Agency, (USEPA, 1999). Synonyms of barite include barium sulphate, barytes, heavy spar, and blanc fixe World Health Organization, (WHO, 2001). Barite is often found as underground ore deposits and is commercially mined in China, USA, India, Morocco, Kazakstan, Mexico, Germany, Iran, Turkey, Canada, and other countries (NRC, 1997). The physical and chemical properties of barite make it useful in a variety of industrial and commercial applications.

2.1 Physical and Chemical Properties

The physical and chemical properties of barite are summarized in Table 1.

Barite exists as colourless to white, orthorhombic dipyramidal powder or crystals (WHO, 2001). Impurities associated with barite include aluminum oxide, arsenic, barium, calcium, copper, fluorine, iron (III) oxide, lead, manganese, silica, strontium sulphate, zinc (WHO, 2001; Nova Scotia Natural Resources, 1992), cadmium (Nelson *et al.*, 1984), mercury (Al.com, 2002), or radium (Phillips *et al.*, 2001), which can change the shade of barite to green, blue, pink, red, brown, or yellow (Nova Scotia Natural Resources, 1992).

Barite is brittle with a hardness of 3 to 3.5 on the Mohs scale (Nova Scotia Natural Resources, 1992) and has a specific gravity of 4.2 to 4.6, which is heavy for translucent minerals (Mineral Galleries, 2003). Barite is practically insoluble in water (USEPA, 1999), organic solvents (CEPA, 2002), and weak acids due to the very strong affinity of barium (Ba^{2+}) for sulphate (SO_4^{2-}) ions (USEPA, 1997). Barite produces no volatile compounds and has no odour (Skylighter, 1986).

2.2 Analytical Methods

No published procedures were found for the quantitative measurement of barite in air, soil, water, or sediment. Generally, published analytical methods measure the concentration of barium and do not provide information on the speciation of barium compounds (WHO, 2001).

The following sections refer to quantitative analytical methods for barium rather than barite.

Air

Methods to measure barium compounds in air include an ashing procedure prior to a flame atomic absorption method (NIOSH, 1987 cited in WHO, 2001) or gravimetric assessment of respirable dust samples (OSHA, 1990 cited in WHO, 2001). Atomic absorption spectroscopy (AAS) has also been used to measure barium in air (Miner, 1969 cited in ATSDR, 1992).

Soil and Oilfield Exploration and Production Waste

Generally, methods to measure barium concentrations in soil samples involve an extraction process followed by analysis of the extract.

A range of different extraction methodologies has been used in the analysis of barium in soil. The methods vary in the amount of barium that they extract, and fall into four general groups (given names here for convenience only):

1. "Extractable" methodologies (*i.e.*, extracting the sample with a neutral solvent), recover principally barium adsorbed to soil particles, present as soluble salts, or bound to organic material. Deeley and Canter (1986) used H_2O, KNO_3, and EDTA, to extract barium from drilling fluids. They found that the amount of barium extracted varied between different drilling wastes and that KNO_3 and EDTA were more effective than H_2O at extracting barium. A method was developed in this project for extractable barium based on an extraction with $CaCl_2$. Details are provided in Section 6.2.2.

2. "Standard acid" methodologies dissolve almost all barium that could become environmentally available, including the forms of barium noted in (1) above (USEPA, 2003b). "Standard Acid" methods are the normal practice when analyzing environmental soil samples for trace metals. USEPA Method 3050B uses repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) to extract elements and metals including barium from sediments, sludges, and soil (USEPA, 2003b). Barium bound to silicate minerals is not normally dissolved by this method. An alternate USEPA method for sediments, sludges, and soil is Method 3051, which uses concentrated nitric acid assisted with microwave heating (USEPA, 2003b). Other "standard acid" methodologies use a mixture of HNO_3 and hydrochloric acid (HCl) also referred to as *aqua regia* digestion.

3. The "Louisiana" Method. The Louisiana Department of Natural Resources (LDNR) has developed an extraction method for "true total barium" specifically for use on oilfield exploration and production waste. The LDNR (1988) method uses HNO_3 at a lower concentration than the USEPA methods, but at a higher ratio of solution to solids (LDNR, 1988). Deuel and Holliday (1998) found that total barium concentrations yielded using the LDNR method were nine times greater than those obtained from the EPA methods. The LDNR method has been revised since the Deuel and Holliday (1998) work (LDNR, 2001a).

4. "Total digestion" methodologies, such as USEPA Method 3052, are used to provide a total decomposition extraction of elements and metals including barium from siliceous, organic, and other complex matrices (USEPA, 2003b). USEPA Method 3052 uses a microwave-assisted acid digestion process.

The extract produced by the above methods can be analyzed by one or more of the following analytical methods: atomic absorption spectrophotometry (AAS), flame atomic absorption spectrophotometry (FLAA), graphite-furnace atomic absorption spectrophotometry (GFAA), inductively coupled plasma - optical emission spectrometry (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS). USEPA Methods include 6010B and 6020. Environment Canada (1999) states that USEPA Method 6010 is the recommended analytical measurement for barium in soil.

Two environmental analytical laboratories in Alberta extract barium from soil using USEPA Methods 3050B, 3051, or modifications thereof followed by ICP-AES or ICP-MS analysis. The method recommended for use in Alberta is presented in Section 6.2.2.

Water
USEPA recommends that the digestion of water and other aqueous samples follow Methods 3005A, 3010A, 3015, or 3020. These methods are based on acid digestion or microwave-assisted acid digestion for analysis of total recoverable, dissolved, or total metals (including barium). These digestion methods are suitable for USEPA Methods 7080A and/or 7081 using FLAA, GFAA, ICP-AES, or ICP-MS.

Two environmental analytical laboratories in Alberta use USEPA Methods 3005A, 3015 or modifications thereof for digestion, depending on the solid content of the aqueous sample. Analyses are conducted using ICP-AES or ICP-MS.

2.3 Production and Uses in Canada

In Canada, barite is commercially produced from mines in Nova Scotia and Ontario (NRC, 2001). An NRC report from 1997 also mentions a commercial barite mine in British Columbia (NRC, 1997). Preliminary estimates of revenue generated from barite production in 2001 were $571,000 for Nova Scotia and $2,200,000 for Ontario (NRC, 2001). Approximately 1% of the barite produced globally was from Canadian mines (USGS, 2000a,b) and approximately 7.3% of global barite reserves are located in Canada (USGS, 2000a) in 1999 and 2000. Approximately 90% of the barite imported into Canada is from the US and approximately 90% of the barite exported from Canada goes to the US (NRC, 1997; USGS, 2000a).

In 2000, approximately 16,062 t of barite was used in Canada (NRC, 2002). Up to 90% of the barite produced is used as a weighting agent in oil and gas drilling muds. Drilling muds are pumped down into the borehole during the drilling of oil and gas wells to lubricate and cool the drill bit, float cuttings out, seal rock strata that are porous, and apply hydrostatic pressure to prevent water from filling the borehole (Nelson *et al.*, 1984). Barite is an excellent drilling mud additive because it is soft, easily crushed and milled, virtually chemically inert, has no magnetic effects, is not abrasive, and has a high specific gravity that can withstand high downhole pressure (Nova Scotia Natural Resources, 1992; WHO, 2001).

The following industries also use barite:

- barium compound manufacturing (barite is used as a source of barium);
- pharmaceutical industry (purified barite, which is radio-opaque, is administered in a "barium milkshake" or enema prior to gastrointestinal x-rays);

- the manufacturing of products including paint, optical glass, bowling balls, ink, linoleum, oilcloth, photographic paper, artificial ivory, cellophane, wallpaper, adhesives, sealants, and polymeric fibres and resins;
- sugar refining;
- television and computer screen manufacturing (barite is used in the faceplates and funnelglass of cathode-ray tubes);
- metal casting (barite is used in the mold-release compounds);
- vehicle manufacturing (barite is used in the friction products for brake and clutch pads);
- chocolate manufacturing (barite can be used as a forming agent);
- paper and rubber manufacturing (barite is used as a filler or a weighting agent);
- heavy concrete production (barite is used as a radioactive shield);
- firework production (barite is used to make the colour green); and,
- pond repair (barite is used to stop leaks in lined ponds that cannot be drained).

The above list was generated using information from the following sources: ADMMR, 2001; Memphis NSTA Regional Convention, 2001; CEPA, 2002; Geobopological Survey, 2003; University of Akron, 2003; and Williams, 2003.

2.4 Levels in the Canadian Environment

Barite is a naturally occurring mineral in the Canadian environment, but anthropogenic sources also release barite to the environment. Most quantitative analytical methods measure total barium concentrations in various media; thus, little information was available on barite concentrations in the Canadian environment. The following sections qualitatively discuss barite levels in the Canadian environment.

2.4.1 Atmosphere

Barite is not volatile (Skylighter, 1986) but may be present in the atmosphere as dust and suspended particles (Environment Canada, 1999). Barite and barium carbonate are the most likely forms of barium found in atmospheric particulate matter (WHO, 2001).

Atmospheric barite may be emitted during anthropogenic activities or as dust originating from barite-containing soil or mining activities (WHO, 2001). Although barite is present in the air where dust levels are naturally high (WHO, 1990 cited in Environment Canada, 1999), the primary source of barium in the atmosphere is from industrial activities (USEPA, 1999). In 1976, the mining and processing of barite ore released approximately 3,200 tonnes of barite to the atmosphere. Fugitive dusts from the use of barite in drilling activities released an additional 100 tonnes of barite (*WHO, 1990 cited in Environment* Canada, 1999). Barite is also emitted to the atmosphere in the exhaust of diesel-powered vehicles. Pierson *et al.* (1981 cited in Environment Canada, 1999) reported approximately 95% of the barium added to diesel fuel was emitted as barite in exhaust.

No information on barite concentrations in ambient air was found.

2.4.2 Indoor Air

Although a proportion of airborne house dust likely contains barite, studies evaluating the concentration of barite in indoor air have concentrated on factory situations. In factories where barite is added to mixing hoppers, barite concentrations of 3.5 to 9.1 mg/m^3 (8-h time-weighted-average) were measured in air. Barite levels in air ranged from 1.3 to 3.7 mg/m^3 in factories where barite ore is processed and from 1 to 3.5 mg/m^3 in factories where plastics and coatings are formulated (WHO, 2001).

2.4.3 Soil

Barite occurs naturally in soil and is precipitated into geological faults when soluble barium minerals (resulting from limestone, feldspar, and/or shale deposit weathering) encounter sulphate solution (WHO, 1990 cited in Environment Canada, 1999). Barite deposits are commonly associated with quartz, fluorspar, calcite, and metallic sulphides and may be present at the soil surface (in outcrops, hillsides, or soil concretions) or deep below the earth's surface (ADMMR, 2001; WHO, 2001). The majority of barite is found in sedimentary formations, as nodules resulting from the weathering of barite-rich sediments, in veins in limestone and sandstone, or in beds (WHO, 2001; Deuel, 2003).

CCME (1999) provides information on the concentration of barium in uncontaminated Canadian soils. Uncontaminated surface soils (n = 1,244) from Quebec had a mean barium concentration of 62 mg/kg. Uncontaminated soils from rural locations in Ontario (n = 101) had a mean barium concentration of 72 mg/kg. All these samples were analyzed using inductively coupled plasma (ICP) or atomic absorption spectrometry (AAS) following HNO_3/HCl digestion. Data from the Prairie Provinces were available, but had been subjected to a much more aggressive digestion technique ($HF/HClO_4/HNO_3$). This digestion technique can release barium from mineral grains, and accordingly the measured barium concentrations were higher (mean = 662 mg/kg; n = 1,273), but could not be compared with standard environmental analyses (which use an HNO_3/HCl or similar digestion), and accordingly were not used here. The mean of the Quebec and Ontario data was 67 mg/kg.

Measured barium concentrations from two background samples from a clay till soil at a site in southwest Alberta were 281 mg/kg and 224 mg/kg, respectively (ESG, 2003b). This site was also the source of the soil used in the studies reported in Appendices I, II, III, and IV.

The mean barium concentration in 1,128 samples of ambient soil from Alberta was 325 mg/kg (Alberta Environment database, Gordon Dinwoodie, *pers. comm*). This value is used here as a reference background soil concentration.

The disposal of waste drilling muds containing barite is the primary anthropogenic input of barite to soil systems (CEPA, 2002). Once a well is completed, drilling muds are often incorporated into the surface soil of the site or placed in lined or unlined earthen pits ("sumps") on-site, and subsequently mixed, buried and covered with subsoil. Water-based and/or hydrocarbon-based (i.e., "invert') drilling muds often contain barite, bentonite, and other additives (Nelson *et al.*, 1984). The barite concentration in drilling mud used for deep wells may exceed 100,000 mg/kg (Deuel, 2003) and was reported for three sources of barite to range from 429, 000 to 509,000 mg/kg (Nelson *et al.*, 1984). The trace elements arsenic, cadmium, chromium, copper, lead, mercury, nickel, and/or zinc were also present in the barite or drill cuttings (Nelson *et al.*, 1984).

Barite supplied by Dynatec Corp. (one of the two main suppliers of barite to the Canadian Oilfield), between 1992 and the present, has typically contained between 87% and 94% barium sulphate, with the majority of the balance being silica. (information supplied by Dynatec Corp. to Chris Meloche, Chevron Canada Resources, 12 February 2003). Maximum levels of trace metals, based on the information supplied, were as follows. Only data for metals for which CCME (1999) has soil quality guidelines are included.

• Arsenic	9 ppm
• Cadmium	0.5 ppm
• Chromium	10 ppm
• Copper	180 ppm
• Lead	6 ppm
• Mercury	5 ppb
• Nickel	10 ppm
• Selenium	0.1 ppm
• Vanadium	6 ppm
• Zinc	16 ppm

All these values are below the CCME (1999) agricultural guidelines, except for copper (guideline = 63 mg/kg).

Soil/drilling fluid mixtures collected from drilling sumps and flare pits throughout Alberta often contain total barium concentrations in excess of the Alberta Tier 1 criterion (AENV, 1994) (600 mg/kg) or CCME (1999) barium guidelines (750 mg/kg for agricultural land use and 2,000 mg/kg for commercial/industrial land use). Soil and soil/drilling fluid samples collected from 5 flare pits, 7 drilling sumps, and 2 oilfield landfarm areas, yielded elevated total barium concentrations up to 20,700 mg/kg. The majority of soil/drilling fluid samples from sumps and flare pits contain barium at concentrations between 1,000 and 4,000 mg/kg (Komex International Ltd, unpublished data).

2.4.4 Water and Sediments

The majority of barium in sediments is in the form of barite (Environment Canada, 1999). Barite may be present in the water column as a suspended solid, but is usually removed by sedimentation (USEPA, 1997).

No information on barite levels in sediments of Canadian water bodies was found.

2.4.5 Drinking Water

Information regarding the barite content of drinking water in Canada was not found. However, treatment systems typically remove suspended matter (including barite) from drinking water. Barite in water can dissolve to release low concentrations of barium and sulphate ions. Health Canada (1990) provides a review of barium concentrations in raw, treated, and distributed drinking water in Canada and CCME (1999) lists barium concentrations in raw water, some Canadian rivers, sediments, and groundwater. The distribution of dissolved barium in drinking water is beyond the scope of the current report.

2.4.6 Biota

Plants and animal tissues are not expected to absorb high concentrations of barite due to its very low solubility; however, barite may be deposited onto plant leaves or be present in the respiratory or digestive systems of animals. Under certain conditions, the solubilization of barite may occur, making barium available to organisms (these processes are discussed in Section 3). Freeman and Deuel (1984) studied the impacts of land treating drilling wastes (containing barite) in wetland areas and reported the tissue from plants growing on closed drilling waste pits yielded up to 4.6 times more barium than the tissue of plants grown in background soil. Deuel (2003) also reported that plants grown in wetland areas where barite-rich oilfield waste had been deposited in pits contained average barium concentrations of 541 mg/kg, whereas plants grown in background wetland soils contained average barium concentrations of 304 mg/kg. In contrast, Nelson *et al.* (1984) conducted a greenhouse study and found barium levels in plants grown on non-saturated background and soil-drilling mud mixtures to be similar.

2.4.7 Oilfield Drilling Muds

The Alberta Research Council (ARC, 1992) conducted a study of the trace metal content of samples of 651 drilling muds. The mean measured total barium concentration in the solids from the drilling muds was 322 mg/kg, though the maximum measured value was much higher at 23,400 mg/kg. On average, invert muds (*i.e.*, diesel-based muds) had a higher total barium content than water-based muds. The analytical method used to determine the total barium concentrations involved a very aggressive digestion technique using a mixture of HHO_3, HCl, and HF in a Teflon bomb in a microwave oven.

ARC (1992) also analyzed saturated paste extracts to investigate the concentrations of soluble trace metals present in drilling muds. The mean barium concentration in saturated pastes from all 651 muds was 0.32 mg/L, while the maximum was 11.4 mg/L. On average, invert muds had a higher saturated paste barium content than water-based muds.

2.4.8 Other

Information on barite or barium (as barite) concentrations in biota used as human food, commercial food, or breast milk was not found. Barite is sometimes added as a forming agent during chocolate manufacturing (Memphis NSTA Regional Convention, 2001) and has been ruled safe for use in the manufacturing of resinous and polymeric coatings in contact with food (CFR 175.000 (b) cited in Highwood Resources Ltd., 2002).

2.5 Existing Guidelines and Criteria in Various Media

No Canadian guidelines that regulate barite levels in air, water, sediment, or soil have been developed. Although risk-based guidelines for total barium concentrations in drinking water (CCME, 1999) and soil (CCME, 1999) exist, they are not considered applicable to barite as they were developed using literature on soluble barium salts (*e.g.*, barium chloride). Soluble forms of barium have very different fate, transport, and toxicological behaviours than those of barite.

2.5.1 Air

The US Occupational Safety and Health Administration (OSHA) workplace permissible exposure limits for barite in air are 15 mg/m^3 for total dust and 5 mg/m^3 for the respirable fraction (OSHA, 2003). The National Institute for Occupational Safety and Health (NIOSH, 1989) recommended exposure limits for barite in air are 10 mg/m^3 for total dust and 5 mg/m^3 for the respirable fraction. Both OSHA and NIOSH limits are time-weighted averages for an 8-h period.

USEPA (2002) Region 9 derived preliminary remediation goals (PRGs) for sites contaminated with barium and/or barium compounds. The risk-based PRG calculated for ambient air is 0.52 µg/m^3 (USEPA, 2002).

2.5.2 Water and Sediments

No barite guidelines for water or sediment were found.

Barium guidelines for drinking water of 1 mg/L were developed by CCME (1999) and Health Canada (1990). The USEPA guideline for barium in drinking water is 2 mg/L (USEPA, 2003a).

2.5.3 Soil

Louisiana developed criteria for total barium levels in exploration and production wastes. Louisiana Statewide Order 29-B (amended) provided a total barium criterion of 20,000 mg/kg for the closure of pits, containing residual oil and gas exploration and production wastes, located on submerged or elevated wetland sites (LDNR, 2001b). Guidelines of 40,000 mg/kg (LDNR, 2001b) and 100,000 mg/kg (LDNR, 1990 cited in API, 1995) were provided for upland land resources and commercial landfill operations, respectively.

Following the study of many more Louisiana sites, Deuel (2003) recommended a barium guideline of 100,000 ppm for the passive closure of sites containing residual oil and gas exploration and production wastes. Deuel (2003) also suggested that the need for a barium guideline was negated for passive closure sites where sufficient agricultural grade gypsum is topically applied as a sulphate source to precipitate the barium ions into barite.

In 1995, the American Petroleum Institute (API) derived risk-based guidelines for barium in exploration and production wastes. A maximum barium concentration of 180,000 mg/kg (measured using the LDNR extraction method for "true total barium") was determined for soil (API, 1995).

USEPA (2002) Region 9 PRGs for sites contaminated with barium and/or barium compounds are 5,400 mg/kg for residential soil and 67,000 mg/kg for industrial soil. Soil barium concentrations of 82 and 1,600 mg/kg were calculated to be protective of groundwater if no dilution or attenuation in the soil occurs, or if a dilution attenuation factor of 20 is present, respectively (USEPA, 2002).

CCME (1999) provides soil quality guidelines for barium that are based on soluble barium compounds. CCME (1999) guidelines for agricultural, residential/parkland, commercial, and industrial land uses are 750 mg/kg, 500 mg/kg, 2,000 mg/kg, and 2,000 mg/kg, respectively.

3. ENVIRONMENTAL FATE AND BEHAVIOR

Barite is not very mobile or bioavailable under most atmospheric, water, or soil conditions and does not undergo photolysis, abiotic, or biotic oxidation to yield barium ions (USEPA, 1991). Information on the environmental fate and behaviour of barite or barium (as barite) is limited because the barium cycle has not been investigated (Baldi *et al.*, 1996). The following sections provide a limited discussion on the environmental fate and behaviour of barite in the environment.

3.1 Atmosphere

Most anthropogenic sources of barium emissions release barium (including barite) in particulate form. Atmospheric barium is not widely dispersed (USEPA, 1997). Although some smaller barium-containing particles may have a residence time in the atmosphere up to a few days, the majority of particles are greater than 10 µm in size and will quickly settle back to land or water (WHO, 2001). The residence time of barium-containing particulates in air is a function of barium speciation, the chemical nature of the particulate, and environmental factors (*e.g.*, rainfall). Barium is deposited to land and water through wet and dry deposition (USEPA, 1997).

3.2 Water and Sediments

Barite solubility is very low in water due to the strong affinity of barium ions for sulphate ions (USEPA, 1991). Because barite readily forms and precipitates out of solution, barium ion availability in water is usually inversely related to the sulphate concentration (WHO, 2001). USEPA (1997) states that natural waters containing barium usually contain enough sulphate and carbonate to precipitate barite and/or barium carbonate. Once formed, barite is very sparingly soluble under neutral pH and oxidized conditions (Carbonell *et al.*, 1999). Deeley and Canter (1986) completed chemical analyses on the aqueous phase of a drilling mud pit and found that while high levels of total barium were present in the sediments, much lower aqueous phase concentrations of barium were present. Barium formed compounds that were insoluble over a wide range of pH values. Deuel (2003) reports the soluble barium released from barite in water increased from 1.38 mg/L (with 1,000 mg barite/L) to 24.9 mg/L (with 1,000 mg barite and 30,000 mg NaCl/L) with the addition of salts. Subsequent addition of 10,000 mg gypsum/L to the barite/salt mixture reduced the soluble barium concentration in water to 0.02 mg/L. The low availability of barium from barite is a function of its low solubility and the high affinity of barium ion for the sulphate ion (USEPA, 1991).

Barite suspended in the water column usually deposits onto the sediments (USEPA, 1997). Suspended barite may be transported for great distances if high flow and low sedimentation rates are present (WHO, 2001).

In anaerobic environments under low sulphate conditions, anaerobic bacteria can enhance barite solubility in aqueous environments and potentially result in increased concentrations of dissolved barium. In nature, anaerobic, sulphur-poor aquatic environments exist, but are often present in northern glaciated regions in isolated water bodies (*i.e.*, not connected to flowing surface and groundwater). These remote aquatic systems are unlikely to release barium ions at concentrations that are ecologically significant (USEPA, 1997).

As barite is dissolved under certain pH, temperature, and dissolved sulphate level conditions, sulphur-reducing bacteria utilise the sulphate, disrupt the dissolved / solid equilibrium and cause more barite to be dissolved. Microbial solubilization of barite in anaerobic environments can release barium ions from barite at concentrations greater than expected based on barite solubility (USEPA, 1997; McCready *et al.*, 1980) and potentially release trace elements co-precipitated in the barite (*e.g.*, arsenic). Some anaerobic microorganisms use dissolved sulphate as an electron acceptor for anaerobic respiration and thus reduce sulphate to sulphite. Barium ions do not form stable compounds with sulphite ions, and accordingly dissolved barium concentrations may increase (USEPA, 1991). Soluble barium, released from barite via the process described above can form other barium compounds (*e.g.*, $BaCO_3$, BaS) (Baldi, *et al.*, 1996), which may be more bioavailable to aquatic organisms than barite itself. McCready *et al.* (1980) found that sterilised sediments, containing no microorganisms, released minimal barium.

Under reducing conditions, barite solubilization has been reported by Huck *et al.* (1989) and Fedorak *et al.* (1986) who investigated the bacterial reduction of barium/radium sulphate sludges in mine tailing pond sediments and the subsequent release of soluble barium to the overlying water. Fedorak *et al.* (1986) found the sulphate-reducing activities of microorganisms in radium mining sludges to be carbon limited and Phillips *et al.* (2001) reported that barium ion release in three oilfield wastes was dependant on the respiration rates of sulphate-reducing bacteria. Carbonell *et al.* (1999) also demonstrated the release of soluble barium from barite in sediments under low pH and highly anaerobic conditions and McCready *et al.* (1980) found soluble barium release from barite in sediments was dependent on pH and the sulphate concentration. The greatest amounts of barium were released at high and low pH (McCready *et al.*, 1980). *Desulfovibrio vulgaris* was identified as the sulphate-reducing organism responsible for metabolising barite in radium mine sludge under neutral pH conditions in anaerobic sediments by McCready and Krouse (1979, cited in McCready *et al.*, 1980) and *Desulfovibrio desulfuricans* was shown to release soluble barium from barite in sewage sludge under anaerobic conditions (Baldi *et al.*, 1996).

While barite solubilization under reducing conditions has been demonstrated, the resulting concentrations of dissolved barium may be lower than expected based on stoichiometry. Phillips *et al.* (2001) reported that while sulphate-reducing bacteria released soluble barium from barite in three different oil-field materials, Ba^{2+} release was not stoichiometric with sulphide production and released less than 0.1% of the barium present in barite. Baldi *et al.* (1996) reported that the concentration of soluble barium released (1.2 mg Ba/L was measured) was well below the concentration expected based on the amount of H_2S evolved (109 mg Ba/L was expected). McCready *et al.* (1980) also found precipitated barite becomes less soluble with age.

3.3 Soil

Barite in soil has very low mobility due to its insolubility (USEPA, 1997) and inability to form soluble complexes with humic and fulvic matter (USEPA, 1984 cited in WHO, 2001). Due to the strong affinity of barium ions for sulphate, the amount of barium present in well-aerated, neutral soils is dependant on sulphate availability (USEPA, 1991). Under non-saturated conditions and natural fluctuations in pH, barite solubility in soil is unlikely to increase (Deeley and Canter, 1986).

Decreased pH, anaerobic conditions, reduced reduction-oxidation potential, elevated chloride or dissolved salt concentrations, and low sulphate concentrations can all result in the solubilization of barite and increased concentrations of dissolved barium in soil (USEPA, 1991; Branch *et al.*, 1990). Deeley (1984; cited in USEPA, 1991) demonstrated that barite can be converted to a soluble barium salt [salt was not specified] in landfills under certain pH and ionic strength conditions. However, soil pH conditions low enough to cause barite solubilization (*i.e.*, pH < 4) are unlikely to occur in oilfield drilling sumps as drilling muds and/or clay textured subsoil (i.e., "mineral soil") have a large neutralizing capacity. Deeley and Canter (1986) concluded that pH changes in the natural environment would not be sufficient to result in the release of soluble barium from barite. Deuel and Freeman (1989) also concluded that in the natural environment, barite poses no risk under oxidative conditions. Redox conditions sufficiently low to solubilize barite (*i.e.*, -200 to -240 mV), are rarely found in mineral soils (API, 1995).

A study by Deuel and Freeman (1989) found that barite in drilling mud remains in the solid phase even after being pumped downhole and subjected to heat and alkaline conditions during drilling. API (1995) stated that in many cases, the addition of barite to soil in the form of drilling fluids may have no effect on barium concentrations in soil solution. Deuel and Holliday (1998) concluded that the majority of barium present in drilling solids is in the residual fraction, which is "inconsequential to barium bioavailability, mobility or reaction in the natural environment". Results from ESG (2003a) (report provided in Axiom Environmental (2004)) showed soluble barium concentrations in pore water within soil-plant microcosms increased over 14 days after barite was added; however, final soluble barium concentrations remained low. The greatest increase was observed for 100% barite, where soluble barium increased from 0.3 to 2.4 mg/L over 14 days. Nelson *et al.* (1984) reported that the barium present as barite in drilling mud was not bioavailable to Swiss chard or ryegrass during a greenhouse study. However, trace elements co-precipitated into barite were available for plant uptake. Nelson *et al.* (1984) concluded that plants took up arsenic, cadmium, copper, lead, nickel, and zinc from drilling fluids.

Deverel *et al.* (1986) state that for sulphate to be reduced to sulphide in soil (and hence for soluble Ba^{2+} to be released from barite), highly reducing conditions are required. Sulphate reduction can occur in soil at a redox potential of approximately –240 mV (Lindsay, 1979 cited in Branch *et al.* 1990), which is considered "much reduced" (Branch *et al.* 1990). Branch *et al.* (1990) evaluated barium solubility in flooded and aerated non-hazardous oilfield waste and found that flooded oil field waste could develop very low redox potentials; however, the presence of high sulphate ions (either present in the oilfield waste originally or added as gypsum) prevented the increase of barite solubility under flooded and high soluble salt conditions. The authors suggest that increased barite solubility could occur under low sulphate conditions.

Deuel and Freeman (1989) demonstrated the conversion of barite to soluble barium in soil under anaerobic conditions in the laboratory. Deverel *et al.* (1986) also reported anaerobic reduction of sulphate to sulphide in three organic soils. In contrast, Deuel and Holliday (1990), Campbell Wells Corporation/K. W. Brown and Associates Inc. (1989; cited in USEPA, 1991), and Branch *et al.* (1990) found that anaerobic conditions did not result in increased barium ion concentrations in drilling mud waste. Deuel and Holliday (1990) conducted their experiment under anaerobic conditions normal for drill pit fluids in the absence of air. USEPA (1991) reviewed these studies and concluded that the anaerobic solubilization of barite can generate soluble forms of barium that may be transported to surface water and groundwater.

Freeman and Deuel (1984) reported that plants grown in barite-rich oilfield waste in wetlands contained more barium than plants grown in wetland background soil; however, the increase in barium content was not significant and barium concentrations in plants were below those shown to adversely affect plant yield (Freeman and Deuel, 1984). In addition, this study found that for one plant species (*Bacopa monnieri*) 65% of the variability in plant barium content could be explained by soil pH; this plant species absorbed less soil barium at a lower pH.

The addition of barite at concentrations up to 300,000 mg/kg was found to increase soil pH and decrease soil moisture; however, conditions remained within the range supportive of plant and soil invertebrate survival and growth. Soil electrical conductivity was not affected by the addition of barite (ESG, 2003a). Miller (1980) reported a slight increase in pH (from 6.0 to 6.7) and a substantial increase in electrical conductivity (0.34 to 1.01 mmhos/cm) upon the addition of bentonite and barite to soil.

Fedorak and Gamez (2003) investigated the effect of different redox conditions on barite solubilization with a matrix of Alberta clay-till soils. Their report is included in this document as Axiom Environmental (2004), and is summarized here. The range of reducing conditions that they considered included aerobic, nitrate-reducing, manganese-reducing, iron-reducing, sulphate-reducing, and methanogenic conditions. Their microcosms included soils contaminated with barite and hydrocarbon (from diesel-based muds disposed of in an old oilfield sump), and background soils either spiked or not spiked with barite. Some microcosms were supplemented with an electron donor (glucose or lactate) while others were not supplemented. The results of this work indicate that:

- Ba^{2+} could be released at low concentrations (typically 3 – 6 mg Ba^{2+}/kg soil, but no more than 14 mg Ba^{2+}/kg soil) from either soil or barite when a supplementary electron acceptor (glucose or lactate) was provided in excess.
- However, in absence of supplementary excess glucose or lactate (as would be the case in the environment), either no Ba^{2+} was released, or no more Ba^{2+} was released from soils spiked with barite than from unspiked soils.

A study commissioned for this project investigated the possible mobilization of barium by salts, and is reported in Axiom Environmental (2004). The purpose of this study was to determine whether a release of oilfield brine could result in the mobilization of barium from barite. The study concluded that the addition of sodium chloride could result in the release of barium from soil; however, sodium chloride did not cause the release of barium from barite.

4. BEHAVIOR AND EFFECTS IN BIOTA

The following section discusses the behaviour and effects of barite in terrestrial biota.

4.1 Soil Microbial Processes

No information was found concerning the effects of barite on soil microorganisms or soil microbial processes. Barite has a very low aqueous solubility and its biological uptake by soil microorganisms is expected to be limited under most natural soil conditions. Under non-saturated conditions and within the typical range of environmental pH values, barite solubility in soil is unlikely to increase (Deeley and Canter, 1986).

Dissolved barium may, however, be released from barite under sulphate-reducing conditions and could then have a negative effect on some soil microorganisms. Intracellular barium concentrations of 0.58 µg/mg (dry weight) were sufficient to inhibit microbial growth in sulphur-reducing bacteria (Baldi *et al.*, 1996). However, information in the scientific literature on the effects of barium to soil microorganisms is limited (CCME, 1999).

4.2 Terrestrial Plants

Although barite may be deposited onto leaves or be present in soil, barite is not considered bioavailable to plants due to its low aqueous solubility (CCME, 1999). Nelson *et al.* (1984) concluded that barium from barite was not available to Swiss chard and ryegrass grown in soil containing drilling fluids and that the concentration of barium in soil/drilling fluid mixtures was not predictive of total of barium concentrations in plant tissues. Freeman and Deuel

(1984) found that plant uptake of barium in wetlands was not dependent on the concentration of measured plant-available barium in the soil/drilling fluid due to temporal variability in available barium measurements.

Soluble forms of barium (released from barite) may become available to plants for uptake under reducing conditions (Freeman and Deuel, 1984; Deuel and Holliday, 1998). The metabolic fate, behaviour, and toxicity of barium in terrestrial plants are discussed by CCME (1999).

4.2.1 Metabolic Fate and Behaviour

Barium is not an essential plant nutrient (Vanselow, 1966 cited in Deuel, 2003). A discussion of the metabolic fate of barite was not possible due to a lack of available information.

4.2.2 Toxicity

Barite Toxicity
Selected toxicological studies on the effects of barite on plants are presented in Table 2.

Toxicity studies by ESG (2003a) indicated that the toxicity of barite to three plant species is very low. Plant toxicity tests for barite were conducted using alsike clover (21-d), orchardgrass (*Dactylis glomerata*; 14 d), and perennial ryegrass (*Lolium perenne*; 14-d) grown in a clay till soil amended with barite to achieve concentrations ranging from 1,000 mg barite/kg dry weight soil to 100% barite (1,000,000 mg barite/kg dry weight soil). Seedling growth was generally unaffected by barite content and the reduced root length in alsike clover observed at 50,000 mg barite/kg dry weight soil during the range finding test was not confirmed in following tests. The only adverse effects observed were the following:

- alsike clover emergence was reduced in 100% barite, but not at lower concentrations;
- alsike clover shoot length was reduced in 100% barite, but not at lower concentrations; and,
- perennial ryegrass root length was reduced in 100% barite, but not at lower concentrations.

Emergence in alsike clover may have been reduced because barite is a very dense growing matrix and not due to a toxicity response (ESG, 2003a). Where possible, linear regression analysis was performed on data to calculate inhibition concentrations (IC). The only IC values that were lower than 100% barite were IC_{20} values of 850,000 and 960,000 mg/kg for root length in orchardgrass and perennial ryegrass, respectively (ESG, 2003a).

Miller *et al.* (1980) added barite to soil to assess potential impacts to the growth of green beans (*Phaseolus vulgaris* L., 'Tendergreen') and sweet corn (*Zea mays* var. saccharata (Sturtev.), 'Bailey' Northrup King-199). To simulate two ways in which barite could be introduced to the soil of an oilfield site, barite was 1) incorporated into soil at 227,500 mg barite/kg air dry soil to imitate barite disposal and disking into the soil and 2) added at a higher concentration (795,300 mg barite/kg air dry soil) to imitate an accidental barite release. Neither barite treatment significantly reduced the growth of aboveground green bean plant parts. A barite concentration of 795,300 mg/kg reduced sweet corn growth by 20%, but a concentration of 227,500 mg/kg had no effect.

Toxicity of Soluble Barium Compounds

The toxicity of soluble barium compounds (chloride and nitrate salts) to plants (peas, green beans, barley) has been investigated by a number of authors. However, the results of these tests are not relevant to this project since barium is expected to be much more available from soluble compounds than from the sulphate. However, Stantec (2003) conducted a study of the toxicity of barium acetate to plants, specifically for this project. The rationale for the study is discussed further in Section 6.2.3, the study itself is reproduced in Axiom Environmental (2004) and the data analyzed in Axiom Environmental (2004). Essentially, the Stantec (2003) study correlates the toxic response from barium acetate to the concentration of extractable barium measured in the soil. Extractable barium is a measure of the barium in solution in the porewater together with the barium sorbed to clay surfaces, and is intended as an approximation of the barium available to plants and soil invertebrates. The methodology for analyzing extractable barium is discussed in Section 6.2.2.

4.3 Terrestrial Invertebrates

4.3.1 Metabolic Fate and Behaviour

No information on the metabolic fate and behaviour of barium (as barite) in soil invertebrates was found.

4.3.2 Toxicity

Barite Toxicity

Selected toxicological studies of barite effects on soil invertebrates are presented in Table 3.

Three studies on four species of terrestrial invertebrate found no adverse effect on survival at the maximum concentrations investigated. Simini *et al.* (2002) investigated the effect of barite on the earthworm *Eisenia fetida*, and found no effect at their highest concentration of 17,000 mg barite / kg soil (10,000 mg barium / kg soil). Kuperman *et al.* (2002) investigated the effect of barite on the Enchytraeid worm *Enchytraeus crypticus*, and found no effect at their highest concentration of 17,000 mg barite / kg soil.

Studies by ESG (2003a) found no effect on invertebrate survival at any concentration. A 14-d acute toxicity test for barite was performed on earthworms (*Eisenia andrei*) and springtails (*Onychiurus folsomi*) using a clay till soil amended with barite to achieve concentrations ranging from 1,000 mg barite/kg dry weight soil to 100% barite (1,000,000 mg barite/kg dry weight soil). No earthworm or springtail mortality was observed during the duration of the test.

Toxicity of Soluble Barium Compounds

The toxicity of soluble barium compounds (acetate and nitrate salts) to terrestrial invertebrates (enchytraeids, earthworms, collembola) has been investigated by a number of authors. However, the results of these tests are not relevant to this project since barium is expected to be much more available from soluble compounds than from the sulphate. However, Stantec (2003) conducted a study of the toxicity of barium acetate to terrestrial invertebrates, specifically for this project. The rationale for the study is discussed further in Section 6.2.3, the study itself is reproduced in Axiom Environmental (2004) and the data analyzed in Axiom Environmental (2004). Essentially, the Stantec (2003) study correlates the toxic response from barium acetate to the concentration of extractable barium measured in the soil. Extractable barium is a measure of the barium in solution in the porewater together with the barium sorbed to clay surfaces, and is intended as an approximation of the barium available to plants and soil invertebrates. The methodology for analyzing extractable barium is discussed in Section 6.2.2.

4.4 Livestock and Wildlife

4.4.1 Metabolic Fate and Behaviour

No information on the metabolic fate and behaviour of barium (as barite) in livestock or wildlife was found. There is no known function of barium in animals (Vanselow, 1966 cited in Deuel, 2003)

4.4.2 Toxicity

No information on the toxicity of barium (as barite) in livestock or wildlife was found.

4.5 Bioaccumulation

No information was available on bioconcentration factors from barite into soil or plants. However, bioconcentration factors for barium in soil into a variety of plants (0.4) and bioaccumulation factors for terrestrial insects (0.2) were developed following a field study with one sampling event (Hope *et al.*, 1996 cited in WHO, 2001).

5. BEHAVIOUR AND EFFECTS IN HUMANS AND MAMMALIAN SPECIES

USEPA (1991) considered barite to be non-toxic as it exhibits no evidence of carcinogenicity, developmental toxicity, reproductive toxicity, neurotoxicity, gene mutation, or chronic toxicity related to exposure. However, the barium ion is toxic if in an available form. The toxicity of the barium ion depends on the solubility of the barium compound. The low toxicity of barite to humans and environmental receptors is largely due to its insolubility in water, weak acids, and alcohols (USEPA, 1991). There is no known function of barium in animals (Vanselow, 1966 cited in Deuel, 2003). Acute and Chronic toxicological studies of barite effects on mammalian species are presented in Tables 4 and 5, respectively.

5.1 Pharmacokinetics

5.1.1 Absorption

Inhalation

In the respiratory system, the solubilization of inhaled barite and subsequent absorption of barium has been demonstrated in a number of studies by clearance of barite from the respiratory system followed by the excretion of barium in urine (Morrow *et al.*, 1964; Cember *et al.*, 1961 cited in Cuddihy and Griffith, 1972) or the presence of barium in the lymph system (Takahashi and Patrick, 1987).

The time taken to clear barite from the respiratory system was found to be proportional to the solubility of barium compounds as inhaled $BaCl_2$ was cleared more readily from the pulmonary system than barite (Cuddihy *et al.*, 1974). Morrow *et al.*, (1964) also found that in the first 24 h after inhalation, the clearance of insoluble dusts from the lungs of beagles increased with dust particle size.

Barite may be cleared from the respiratory tract by mucociliary mechanisms, lung-to-blood transfer, or potentially, solubilization in the tracheal wall. In beagle dogs, approximately 75% of inhaled barite was deposited to the pulmonary region and 25% entered the tracheobronchial and nasopharyngeal regions (Cuddihy *et al.*, 1974). In rats, 15.3% of intratracheally-injected barite was cleared from the lungs within 24 h; 7.9% was cleared by mucociliary mechanisms (removed material is eventually swallowed) and 7.4% was cleared by lung-to-blood transfer. The half time for each process was 9 h during the first 24 h after administration (Spritzer and Watson, 1964). Barite inhaled at a concentration of 1.1 µg/L for 30 to 90 min had a biological half time of 8 days in the lower respiratory tract of beagles (Morrow *et al.*, 1964). Takahashi and Patrick (1987), intratracheally administered barite to the caudal trachea (injection site). After 7 days 0.41% of the original barium dose remained; 10 times this level of barium had moved to the cranial region of the trachea. After 7 days, barite was cleared exponentially from the caudal trachea with a half time of 88 days and from the cranial trachea with a half time of 66 days. Barium in the trachea of rats was detected up to 6 months after injection. Takahashi and Patrick (1987) concluded it was possible that some of the barite had been cleared from the tracheal wall by solubilization, but barium concentrations in blood, and the entire carcass (including skeleton) were found to be low. USEPA (1991) stated that in rats following intratracheal installation, approximately 1.3% of the barium in barite was absorbed via solubilization (USEPA, 1991).

In the body, barite particles are often engulfed by macrophages, which do not appear to suffer any toxic effects at a dose of 10 barite particles per macrophage cell (Rae, 1977). Macrophages have been reported to engulf barite particles in the lungs (Parkes, 1982 cited in BIBRA, 1994), tracheal wall (Stirling and Patrick, 1980, cited in Takahashi and Patrick, 1987), and gastrointestinal tract (Najjar *et al.*, 1973 cited in Shackleton *et al.*, 2000; Shackleton *et al.*, 2000). Takahashi and Patrick (1987) were not able to determine whether barite particles retained in the trachea by macrophages had become solubilized *in situ*.

Oral

Barite solubilization and subsequent barium absorption has been demonstrated from the gastrointestinal tract as evidenced by increased barium concentrations in blood and urine. A single, low dose of barite (0.5 mg/kg body weight) given to rats by gavage was absorbed at an equivalent rate to a similar concentration of $BaCl_2$ (a much more soluble barium salt). McCauley and Washington (1983) attribute the absorption of barium at low barite concentrations to solubilization in the gastrointestinal tract by stomach acids.

When large doses of barite are administered orally, only a very small fraction of the barite is solubilized by gastric hydrochloric acid and absorbed (*e.g.*, prior to gastrointestinal x-ray). However, barium levels in the blood and urine of humans were found to increase significantly following ingestion of 58 to 400 g of barite in radio-opaque contrast materials (Mauras, *et al.*, 1983, Claval, *et al.*, 1987 cited in USEPA, 1999). Barite solubilization, which led to the fatal barium poisoning of a patient who had an impaction of the colon following the oral administration of barite, may have occurred due to cationic exchange in the intestinal tract where barite was converted into barium chloride and absorbed by the intestinal mucosa (Pélissier-Alicot *et al.*, 1999). Elevated barium concentrations were observed in the blood and cerebrospinal fluid of this patient.

Dermal

No information on the dermal absorption of barite was available found, but dermal absorption of barite is not expected (USEPA, 1991).

5.1.2 Distribution

Inhalation

In the body, the greatest concentrations of barium are found in the skeletal system (USEPA, 1999) and absorbed barium from inhaled barite has been measured in the skeletal system. Cuddihy *et al.* (1974) reported approximately 25% of absorbed barium (from inhaled barite) was transported to the skeleton. In contrast, Takahashi and Patrick (1987) found that only 0.84% of the barium (as barite) injected into the tracheas of rats, was present in the carcass (including the skeleton) after 7 days, 1.3% was present after 4 weeks, and 1.1% was present after 24 weeks. Differences in the results of these two studies may have been due to the preparation and resulting solubility of barite particles (Takahashi and Patrick, 1987). Within 10 days of an intratracheally-injected dose of barite, rats excreted approximately 5% of the initial dose in urine (Cember *et al.*, 1961 cited in Cuddihy and Griffith, 1972).

Takahashi and Patrick (1987) demonstrated that barite administered to the tracheal lumen drained to the lymph nodes in the thoracic cavity and the deep and superficial cervical regions, but the proportion of barium cleared from the trachea through these routes was impossible to quantify. Alveolar macrophages can also remove barite from alveoli in the lungs to regional lymph nodes (Buschman, 1991).

Oral
McCauley and Washington (1983) compared the absorption and distribution of barium from barite and $BaCl_2$ (administered orally at low doses) to rats over a 2-hour period. Similarities in barium absorption and distribution to the eye 2 hours after exposure led them to conclude that the distribution of barium from both compounds to heart, liver, kidney, and skeletal muscle was similar. Measured concentrations of absorbed barium in the heart, eye, skeletal muscle, and kidneys were greater than the whole blood concentration, which suggests barium may concentrate in soft tissue.

5.1.3 Metabolism

No information on the metabolism of barite was found.

5.1.4 Elimination

Absorbed barium from inhaled barite is primarily excreted from the body in feces and urine. Cuddihy *et al.* (1974) found approximately 75% of absorbed barium (from inhaled barite) was excreted in the urine and feces over a 2 wk period by beagles. Approximately 6% of the initial lung deposit of intratracheally-injected barite was excreted in the urine of rats (Spritzer and Watson, 1964). Cember *et al.* (1961 cited in Cuddihy and Griffith, 1972) found that within 10 days of an intratracheally injected dose of barite, rats excreted approximately 5% of the initial dose in urine. Morrow *et al.* (1964) reported that barium (from inhaled barite) was so rapidly excreted in the feces and urine of beagles within one week of exposure, the study of barite elimination over a long period of time was not possible.

Some barium taken up by organisms may accumulate in their body tissues. Bioaccumulation factors for white-footed mice (0.02) and hispid cotton rats (0.02) were developed following a field study with one sampling event. Barium was lost from these receptors at a fairly rapid rate and depuration rates of 0.4/day and 0.2/day were estimated for white-footed mice and hispid cotton rats, respectively (Hope *et al.*, 1996 cited in WHO, 2001).

5.1.5 Modifying Factors

No modifying factors for barite pharmakokinetics were found.

5.2 Mammalian Toxicology

Acute and chronic toxicological studies of barite effects on mammalian species are presented in Tables 4 and 5, respectively.

5.2.1 Acute Toxicology

Inhalation

A single intratracheal dose of 0.6 ml/kg Ba147 (a preparation containing 85% barite) to rabbits did not cause treatment-related effects to pulmonary ventilation, blood gas levels, or lung weights up to 4 weeks after administration. Dose related shadows were observed in lungs and 78% of animals exhibited bronchopneumonia, bronchitis, or bronchiolitis one week after dosing. Further details on this study were not available as USEPA was only able to obtain an English abstract (Uchiyama, *et al.*, 1995 (abstract) cited in USEPA, 1999). Stirling and Patrick (1980, cited in Takahashi and Patrick, 1987) reported that 1 to 7 days after barite was injected into the trachea of rats, barite particles retained in the tracheal wall were located within macrophages.

Oral/Gastrointestinal Tract

Boyd and Abel (1966) administered barite to rats through an intragastric cannula. No deaths were caused by dosages up to 160 g barite/kg body weight in a pilot test. Definitive tests found a single intragastric administration of barite to rats at doses of 25 to 40% body weight caused death by stomach rupture or bowel obstruction, intestinal bleeding and generalized blood clotting. The LD_{50} for stomach rupture was 307 +/- 29 g barite/kg body weight and for bowel obstruction was 364 +/- 41 g barite/kg body weight (Boyd and Abel, 1966).

5.2.2 Sensitization and Irritancy

Barite may cause brief inflammatory responses or ulcers in wounds. The intestinal tissue of 20 dogs exhibited an inflammatory response (especially by neutrophilic granulocytes) when exposed to barite. Once the barite had been engulfed by macrophages, the inflammatory response rapidly decreased (Najjar *et al.*, 1973 cited in Shackleton *et al.*, 2000). In dogs, ulceration was observed when a deep colorectal biopsy was immediately followed by a barium enema; however, the healing process was comparable to wounds with no barite added (Maglinte *et al.*, 1983 cited in Shackleton *et al.*, 2000). Shackleton *et al.* (2000) reported no effect of barite application on wound healing, granuloma formation, weight gain, activity, and survival of rats with gastrointestinal wounds (stomach, small bowel and colon).

5.2.3 Short-Term Toxicity/Subchronic Toxicity

No information on the subchronic toxicity of barite was found.

5.2.4 Immunotoxicity

No information on the immunotoxicity of barite was found.

5.2.5 Long-Term/Chronic Toxicity

Inhalation

Holusa *et al.* (1973, cited in BIBRA, 1994) exposed rats to atmospheres containing 40 mg/m^3 barite for 5 h/day for up to two months. Following barite exposure, the lungs and associated lymph nodes were examined at a microscopic level. Holusa *et al.* (1973, cited in BIBRA, 1994) described inhaled barite as an "inert dust" based on the pattern of tissue changes observed in the lungs.

Oral

To determine whether heavy metals (including barium) could be safely used as nutrient markers, Hutcheson *et al.* (1975) fed barite, scandium, chromium, lanthanum, samarium, europium, dysprosium, terbium, thulium, and ytterbium oxides to three generations of mice. The barite concentration of this mixture ranged up to 8 ppm (approximately 1.2 mg/kg bw/day). The body weight gain, mortality rate, reproduction, lactation, and general appearance of mice in the 8 ppm barite treatment, and the gross appearance of organs and blood chemistry of mice in the 0.8 ppm barite treatment were not overtly affected by barite consumption at these levels.

5.2.6 Carcinogenicity

Studies on the potential carcinogenicity of barite were not available for any route of exposure; however, NTP (1994) found no evidence of carcinogenic activity of barium chloride dihydrate in rats or mice exposed for 2 years via drinking water. Barium chloride dihydrate is more soluble than barite and so the barium bound in barium chloride dihydrate is expected to be more bioavailable to organisms. Extrapolation of the NTP (1994) study results to barite lead to the conclusion that barite is not likely a carcinogen in mammals. A toxicity review by USEPA (1991) found no evidence for cancer associated with barite exposure.

A local cancer (mesothelioma) developed in 1 of 30 treated rats which had barite injected directly into the pleural cavity (Wagner *et al.*, 1973 cited in BIBRA, 1994).

5.2.7 Mutagenicity

The Registry of Toxic Effects of Chemical Substances (NIOSH, 1994 cited in BIBRA, 1994) describes barite as a mutagen because chromosome damage was reported for mice that received an intraperitoneal injection of 12.5 mg barite/kg bw. However, "the study report in question is in Chinese (Laifu *et al.*, 1986), but the English abstract refers to the testing of a beryllium compound and not barium sulphate" (BIBRA, 1994).

5.2.8 Neurotoxicity

No information on the neurotoxicity of barite was found.

5.2.9 Reproduction

Three generations of mice fed barite at concentrations up to 8 ppm (approximately 1.2 mg/kg bw/day) along with a number of lanthanide metal oxides, showed no effects on the number of females having litters, average litter size, or average weaning weight (Hutchenson *et al.*, 1975).

5.2.10 Teratology

No information on the teratology of barite to mammalian species was found during the preparation of this report.

5.3 Human Toxicology

5.3.1 Acute Toxicology

Inhalation

The aspiration of small amounts of barite into lungs is almost always inconsequential (Ott and Gelfand, 1983) and has led to its use as a bronchiographic contrast agent. Buschman (1991) reported that barite suspensions, administered during bronchography, were retained for years in the lungs through alveolarization and the likely incorporation of barium into macrophages. Retained barite was present in the peripheral airways and alveoli, beyond the ciliated mucosa, and could not be effectively cleared from the lungs. This residual barite did not cause any fibrotic pulmonary changes or toxicity problems after 6 months of retention, but interfered with subsequent imaging procedures (Bushchman, 1991).

Injection

During administration of barite as a contrast agent, perforation of the esophagus, stomach or duodenum may allow the extravasation of barite to into the mediastinum or peritoneal cavity, and result in mediastinitis with an accompanying bacterial infection and peritonitis, respectively (Ott and Gelfand, 1983). Enema complication may also inject barite into the bloodstream where extensive blood clotting in the lungs and heart could potentially be fatal (Ansell, 1976; Blom *et al.*, 1983; Juler *et al.*, 1975; Seaman and Wells, 1965 cited in BIBRA, 1994) or into the vagina causing venous intravasation or vaginal rupture with extravasation (Ott and Gelfand, 1983).

Oral

Boyd and Abel (1966) extrapolated the results of their experiments on albino rats and concluded that humans could not orally ingest enough barite in a single dose to be acutely toxic. However, impaction of the colon has been reported in some case studies following the oral ingestion of large doses of barite prior to gastrointestinal x-ray (USEPA, 1991) and may be fatal (Pélissier-Alicot *et al.*, 1999). Pélissier-Alicot *et al.* (1999) describe a case study where an unknown intestinal occlusion caused impaction of the colon and led to the intravasation or venous embolization of barite, which had been ingested prior to gastrointestinal x-rays.

5.3.2 Sensitization and Irritancy

The impaction of barite particles into the skin, resulting from the explosion of golf balls, may cause the formation of granulomas (a mass or nodule of chronically inflamed tissue produced in response to foreign particles) (Honda and Nishi, 1978 and Ishii *et al.*, 1982 cited in BIBRA, 1994). Imperfectly administered enemas have also been reported to cause rectal granulomas, barium inflammation of the abdomen, and embolization (*e.g.*, Beddoe *et al.*, 1954; Carney and Stephens, 1973; Weitzner and Law, 1972 cited in BIBRA, 1994).

Low incidences of severe adverse reactions have been reported by a number of researchers following the administration of barite meals or enemas. These severe reactions are listed by BIBRA (1994) and include respiratory difficulties, generalized skin rash, abrupt decreases in blood pressure, and fatalities. Most researchers concluded these hypersensitivity reactions were not caused by barite, but by other components in the barite suspension although this hypothesis was rarely confirmed (BIBRA, 1994)

5.3.3 Short-Term/Subchronic Toxicity

Repeated use of barite as a contrast agent prior to gastrointestinal x-rays has little physiological effect other than volume and fluidity changes (Ott and Gelfand, 1983).

5.3.4 Immunotoxicity

No information on the immunotoxicity of barite to humans was found during the preparation of this report.

5.3.5 Long-Term/Chronic Toxicity and Carcinogenicity

Inhalation

Chronic inhalation of barite dust causes baritosis (a benign pulmonary reaction) characterized from x-rays by dense, discrete, small opacities. These opacities are barite particles not tissue lesions (USEPA, 1991). Macrophages engulf the particles and negligible effects to the lung tissue occur (Parkes, 1982 cited in BIBRA, 1994). Baritosis does not have any symptoms and does not decrease pulmonary function (USEPA, 1991). Once exposure has ended, opacities slowly reduce (Doig, 1976).

No studies that addressed the potential carcinogenicity of barite to humans were found during compilation of this report. Barium is classified as Group D – not classifiable to human carcinogenicity by USEPA (1999) because animal studies have not adequately addressed barium carcinogenicity through the inhalation route of exposure.

5.3.6 Neurotoxicity

Neurological and cardiovascular effects were observed following the barium poisoning of a patient who had been orally administered barite prior to gastrointestinal x-rays. An intestinal occlusion of unknown origin caused the barite to remain in the digestive tract. Convulsive seizures and encephalopathy were observed prior to death; these symptoms have been described by previous reports of barium poisoning (Pélissier-Alicot *et al.*, 1999).

5.3.7 Reproduction

No information on potential human reproduction effects from barite exposure was found during the preparation of this report.

5.3.8 Teratology

No information on the teratology of barite to humans was found during the preparation of this report.

5.3.9 Epidemiology

No information on the epidemiology of barite exposure for humans was found during the preparation of this report.

5.4 Human Tolerable Daily Intake

Much of the available data suggest that barite is likely to be significantly less toxic to humans than soluble forms of barite. However, none of the available studies were suitable for developing a human tolerable daily intake (TDI) for barite. In the absence of such studies, the very conservative assumption is made that the TDI for barite is the same as for soluble forms of barium.

The toxicology of barium has been reviewed by Health Canada and the USEPA. Health Canada have developed a Canadian Drinking Water Quality Guideline of 1.0 mg/L (Health Canada 1990), based on an epidemiological study of barium in drinking water by Brenniman and Levy (1984). However, Health Canada has not developed a TDI for barium. The USEPA (1999) has reviewed the toxicology of barium and developed a reference dose (equivalent to a TDI) of 0.07 mg/kg-bw/day, based on a weight-of-evidence approach that focuses on four coprincipal studies: the Wones *et al.* (1990) experimental study in humans, the Brenniman and Levy (1984) epidemiological study, and the subchronic and chronic rat studies that employed adequate diets and investigated both cardiovascular and renal endpoints (NTP, 1994). The McCauley *et al.* (1985) study of unilaterally nephrectomized rats was used to support the identification of the kidney as a cocritical target.

The USEPA TDI (reference dose) of 0.07 mg/kg-bw/day was used in this project to assess safe levels for human exposure.

5.5 Human Exposure

Insufficient information was available to assess the typical exposure of the Canadian population to barite. However, Health Canada (1990) has assessed the exposure of the Canadian population to barium. According to Health Canada (1990) intake of barium through inhalation would be negligible compared with the amount ingested and the primary exposure to barium is through food, with drinking water being significant in areas where barium levels in drinking water are high (0.6 mg/L). Health Canada (1990) estimated the mean daily intake of barium to be slightly more than 1 mg/day, which corresponds to an estimated daily intake of 0.014 mg/kg-bw/day for a 70.7 kg adult.

6. SPECIAL CONSIDERATIONS IN THE DEVELOPMENT OF BARITE GUIDELINES

6.1 Introduction

Typically guidelines for metals are developed for a single element (*e.g.*, barium), rather than a compound such as barite. Developing guidelines for inorganic compounds presents particular challenges, some of which are discussed below.

Barite is highly insoluble, and as such is expected to be largely unavailable. Accordingly, barite can be managed safely in the environment at higher concentrations than would be appropriate for soluble barium compounds. However, while barium analysis is routinely carried out on environmental soil samples, there is no commercial analytical method presently available to analyze specifically for barite.

A typical situation at upstream oil and gas sites (and potentially other sites where barite has been used) is that barium is present at levels above generic guidelines, but, based on the site history, the source of the barium is assumed to be barite. Before barite guidelines can be applied at such a site, it will be necessary to demonstrate:

i. that the barium is still in an immobile/unavailable form; and,
ii. that the barium would not be expected to become more mobile/available in the future.

Studies were commissioned as part of the current project to help resolve these two issues. The studies and their application is discussed in Sections 6.2 and 6.3.

6.2 Barium Speciation

As noted above, there is no commercially available method for determining the speciation of barium in a soil sample. However, the question of whether the available barium in a soil sample is above or below the level that might cause adverse effects can be addressed by correlating the toxic response in plants and invertebrates with the level of available barium. The selection of an analytical method for available barium is discussed in Section 6.2.1. The recommended method for analysis of extractable barium is provided in Section 6.2.2. The toxicity testing program using barium acetate is discussed in Section 6.2.3.

6.2.1 Available Barium

Three techniques for barium analysis were investigated to determine which of them would provide good correlations between the amount of barium measured and the toxic responses of the plants or invertebrates to barium acetate (see following Section). The three techniques were:

1. conventional soil analysis for barium involving an acid digest, followed by inductively coupled plasma – optical emission spectrometry (ICP-OES) detection;
2. ICP-OES analysis of a saturated paste extract; and,
3. ICP-OES analysis of the extractable barium (see Section 6.2.2).

Conducting these analyses on samples from the toxicological work reported in Appendices III and IV indicated that neither technique 1 nor technique 2 was a good predictor of toxic response in plants or invertebrates. Technique 1 appeared to respond to barium that was not available to the biota (Axiom Environmental, 2004), presumably because barium was released from mineral grains during the acid digest. Technique 2 did not respond to barium that was causing a toxic response in plants (Axiom Environmental (2004)), presumably because barium sorbed to clay surfaces is available to plants but is not measured by the technique.

Initial tests of technique 3, however, indicated a much better correlation between analytical results and toxicological response. Accordingly, it was determined that extractable barium was likely to be the best measurement of available barium. John Ashworth and Warren Greig of EnviroTest Laboratories in Edmonton and Saskatoon developed an analytical methodology for extractable barium, respectively. Method development was conducted in two phases. The first phase involved a number of trials to select the most effective extraction methodology. The technique presented in the following Section (6.2.2) was developed to maximize recovery of sorbed barium, and to minimize ICP-OES interference from high concentrations of cations in the extractant. The second phase of method development involved ruggedness testing on the proposed analytical method to determine to what extent the method might be sensitive to small changes in technique. The results of ruggedness testing are included in Axiom Environmental (2004), and indicate that the methodology presented below is suitably rugged.

6.2.2 Analytical Method for Extractable Barium

The recommended procedure for determination of extractable barium (see also Axiom Environmental (2004)) is as follows:

- Prepare 0.1 M calcium chloride (14.7 g of pure dihydrate per litre of de-ionized water).
- Use a representative sub-sample of air-dried, <2 mm soil, weighing at least 10 g.
- Add 0.1 M calcium chloride at a 1:10 ratio, *e.g.*, 10 g soil plus 100 mL, leaving adequate headspace for agitation.
- Agitate for at least 2 h on either a platform or end-over-end shaker.
- Filter the extract either by gravity or under suction, using a filter paper fine enough to yield a clear filtrate.
- Make up standard solutions (*e.g.* of barium acetate or chloride) either in DI water or in the extracting solution.
- Analyze the filtrate and standards by ICP within 24 h.

6.2.3 Barium Acetate Toxicity Testing

A program of acute and definitive toxicity testing using barium acetate with three species of plants and two species of invertebrates was commissioned for this report (Stantec, 2003; Axiom Environmental (2004)). The purpose of this testing was to correlate the toxic response observed in plants and soil invertebrates with the concentration of extractable (*i.e.*, available) barium. It should be noted, however, that the results in Axiom Environmental (2004) are all presented in terms of nominal concentrations (*i.e.*, the mass of barium acetate added), rather than the mass of barium in the sample.

Axiom Environmental (2004) presents the analysis of selected samples from the toxicity testing program for extractable barium, the regression of extractable barium analysed against mass of barium acetate added, and the recalculation of the toxicity testing results expressed in terms of measured concentrations of extractable barium.

6.3 Barium Mobilization

Two studies were commissioned through the Microbiology Department of the University of Alberta, supervised by Dr. Phil Fedorak, to help determine whether the possibility of future mobilization of barium from barite was a significant concern.

6.3.1 Soil Microcosm Study

This study (Fedorak and Gamez, 2003, included in Axiom Environmental (2004) and discussed in Section 3.3) investigated the possible mobilization of barium from barite as a result of microbial degradation, particularly the action of sulphate-reducing bacteria (SRB) on barite. Previous studies had shown that SRBs could mobilize barium from barite under favourable conditions, but none of the studies had used conditions that were particularly relevant to typical upstream oil and gas sites in Canada.

Fedorak and Gamez (2003) showed that while SRBs were capable of mobilizing barium from barite when a supplementary electron acceptor (glucose or lactate) was added, no barium was mobilized from barite in the absence of added glucose or lactate. In particular, the SRBs in these experiments did not seem able to utilize the contaminant hydrocarbon (weathered diesel fuel) in the soil as an electron acceptor. Even in the experiments where glucose or lactate was added, the amount of barium released from barite was relatively small (in all cases less than 12 mg barium released from barite per kg of soil, even though samples were spiked with 2,000 or 10,000 mg of barite per kg of soil).

Overall, it is not anticipated that barium mobilization due to bacterial activity will be a concern at typical upstream oil and gas sites.

6.3.2 Salt Mobilization Study

This study (included in Axiom Environmental (2004) and discussed in Section 3.3) investigated whether salt (such as that associated with a release of oilfield brine) could result in the mobilization of barium from barite. The study found that while salt could release (naturally occurring) barium from soils, no barium was released from barite. Accordingly, barium mobilization from barite due to interactions with salt will not be a concern.

7. DERIVATION OF ENVIRONMENTAL SOIL QUALITY GUIDELINES

Environmental soil quality guidelines were developed for five standard land uses (AENV, 2001): natural areas, agricultural, residential/parkland, commercial and industrial. The last four are the standard CCME (1996) land uses. Natural area land use was introduced by AENV (2001) to reflect areas where none of the four CCME (1996) land uses were applicable, and where regular human occupation was not anticipated. Guidelines for natural areas are similar to those for agricultural land use, but the human health guidelines are not expected to be limiting (based on only occasional human exposure) and are not included in this land use.

7.1 Soil Quality Guidelines for Soil Contact

Two soil quality guidelines for soil contact are developed. The first is based on the concentration of extractable barium and is used as a screening tool to determine whether the barite guideline can be applied. The second is the guideline for barite, and is based on the total barium concentration. Guideline application is discussed in Section 10.

7.1.1 Extractable Barium

The development of the soil contact guideline for extractable barium is described in detail in Axiom Environmental (2004), and summarized here.

Soil sample concentration series from either the beginning or end of five different toxicity tests from the Stantec (2003) program were analyzed for extractable barium. Since all five barium acetate/extractable barium curves essentially formed part of the same data set, it appeared that the measured concentration of extractable barium was independent of the species that was present in the toxicity test, and whether the sample was collected at the beginning or the end of the test. Accordingly, all five data sets were combined before generating a regression equation. A linear regression was fit to the data (see Axiom Environmental (2004) for details).

All the toxicity data in Stantec (2003) (Axiom Environmental, 2004) were presented on a nominal barium acetate basis. In the present document, the 20th percentile effect data (*i.e.*, IC_{20}, EC_{20}, and LC_{20}) were used to compute the soil contact guidelines. The nominal 20th percentile data from Stantec (2003) were converted to an extractable barium basis by applying the regression equation to the nominal toxicity data. The different growth endpoints for each plant species were considered redundant, as were the different reproduction endpoints for springtails. Each group of redundant endpoints was combined as their geometric mean. The soil contact guideline for natural areas, agricultural land, and residential/parkland land uses was calculated as the 25th percentile of the geometric means, and was 260 mg/kg (Table 6). The soil contact guideline for commercial and industrial land uses was calculated as the 50th percentile of the geometric means and was 440 mg/kg (Table 6).

7.1.2 Total Barium Guideline for Barite Sites

If extractable barium concentrations at the site comply with the applicable extractable barium criterion, then the total barium at the site may be assessed against the total barium guideline for Barite Sites.

The soil quality guideline for total barium at Barite Sites (see Section 10 for definition) is based on the barite plant and soil invertebrate studies discussed in Sections 4.2 and 4.3. Most studies found minimal effects on either plants or soil invertebrates. The soil quality guideline for total barium at Barite Sites is conservatively set at the no observed adverse effect level (NOAEL) from the ESG (2003a) definitive plant tests on alsike clover and perennial ryegrass. Both these tests had a NOAEL of 300,000 mg barite per kg soil, which corresponds to 180,000 mg barium per kg soil (Table 6).

7.2 Soil Quality Guidelines for Soil and Food Ingestion

Insufficient data were available to calculate the food ingestion part of this guideline. However, it is not anticipated that bioconcentration of barite into plants will be significant. Nelson *et al.* (1984) reported that the barium present as barite in drilling mud was not bioavailable to Swiss chard or ryegrass during a greenhouse study. Freeman and Deuel (1984) reported that plants grown in barite-rich oilfield waste in wetlands contained more barium than plants grown in wetland background soil; however, the increase in barium content was not significant.

Accordingly, and consistent with AENV (2001), a guideline (SQG_{ESI}) was calculated for ecological soil (but not food) ingestion for natural area and agricultural land use. A meadow vole was selected as the most exposed organism for both land uses, based on soil ingestion rate and body weight. The following equation was used:

$$SQG_{ESI} = \frac{0.75 \times DTED \times BW \times 1{,}000}{SIR \times BF}$$

Where:

SQG_{ESI} = ecological quality guideline for soil ingestion livestock/wildlife (mg/kg);

0.75 = factor to prevent animals from being exposed to more than 75% of the DTED (unitless; CCME, 1996);

DTED = daily threshold effect dose for meadow vole (15 mg/kg-bw/day; see Section 7.2.1 below);

BW = body weight for meadow vole (0.017 kg; USEPA, 1993);

1,000 = conversion factor from kg to g;
SIR = soil ingestion rate for meadow vole (0.058 g/day; see Section 7.2.2 below); and,
BF = bioavailability factor (1; assumed).

Substituting these values in the above equation and rounding to 2 significant figures yields a value of 3,300 mg/kg, which is the guideline for ecological soil ingestion (SQG_{ESI}; Table 6).

7.2.1 Daily Threshold Effect Dose

The daily threshold effect dose (DTED) for barite as barium was calculated for meadow voles. Based on the CCME (1996) protocol, the DTED was calculated using the following equation:

$$DTED = \frac{Lowest\ LOAEL}{UF}$$

Where: DTED = daily threshold effect dose for barite as barium for meadow voles (mg/kg-bw/day);
Lowest LOAEL = lowest "lowest observed adverse effect level (LOAEL)" (75 mg/kg-bw/day – from the NTP (1994) chronic rat study for the endpoint of increased kidney weight); and,
UF = uncertainty factor (5 – CCME, 1996).

Substituting these values in the above equation yields a value for the DTED of 15 mg/kg-bw/day.

7.2.2 Soil Ingestion Rate

The soil ingestion rate was calculated using the following equations:

$$SIR = FIR \cdot PSID$$

and

$$FIR = 0.0687 \cdot BW^{0.822} \cdot 1{,}000$$

Where: SIR = soil ingestion rate for meadow voles (g/day);

FIR = food ingestion rate for meadow voles (2.41 g dry weight/day; calculated below);
PSID = percent soil in diet for meadow voles (2.4% – USEPA, 1993);
BW = meadow vole body weight (0.017 kg – minimum value from the range given in USEPA, 1993); and,
1,000 = conversion factor from kg to g.

Substituting these values in the above equation yields value for FIR of 2.41 g dry weight/day and for SIR of 0.058 g day.

7.3 Nutrient and Energy Cycling Check

This guideline was not calculated due to a lack of adequate data.

7.4 Off-Site Migration Check

The off-site migration check for environmental endpoints is calculated to ensure that wind and water erosion of contaminated material from an industrial site could not cause unacceptable contaminant concentrations on an adjacent residential property (CCME, 1996). The check is calculated using the equation provided in CCME (1999):

$$C_i = \frac{\{[D_m \cdot C_m] - [(D_m - D_d) \cdot BSC]\}}{D_d}$$

Where:

C_i = off-site migration check (mg/kg);
D_m = mixing depth (2 cm, CCME, 1999);
C_m = soil contact guideline for residential/parkland use (180,000 mg/kg, see Table 6);
D_d = depth of deposited material before mixing (0.14 cm; CCME, 1999); and,
BSC = background concentration of the contaminant in the receiving soil (325 mg/kg, Section 2.4.3).

Substituting these values in the above equation gives a value of greater than 1,000,000 mg/kg. This indicates that this check will never be a concern, and the guideline for this pathway is noted as "NA" in Table 6.

7.5 Groundwater Check (Aquatic Life)

This check was not calculated because it is not applicable to metals (CCME, 1999).

7.6 Data Gaps

The available data were sufficient to develop soil contact guidelines both for total barium and for extractable barium.

Data on the effects of barite on soil microbial communities would be required to calculate the nutrient cycling check. However, based on the chemical inertness of barite, it is not anticipated that significant adverse effects would be found.

Further data on the bioaccumulation of barium from barite into plants would be required to calculate the food ingestion part of the livestock/wildlife soil and food ingestion guideline. However, based on the assumed low bioavailability of barite it is not anticipated that bioconcentration of barite from soil into plants would be significant.

8. DERIVATION OF HUMAN HEALTH SOIL QUALITY GUIDELINES

Environmental soil quality guidelines were developed for five standard land uses (AENV, 2001): natural areas, agricultural, residential/parkland, commercial and industrial. The last four are the standard CCME (1996) land uses. Natural area land use was introduced by AENV (2001) to reflect areas where none of the other four CCME (1996) land uses were applicable, and where regular human occupation was not anticipated. Guidelines for natural areas are similar to those for agricultural land use, but the human health guidelines are not expected to be limiting (based on only occasional human exposure) and are not included in this land use.

8.1 Soil Ingestion Guideline

The guideline for this exposure pathway was calculated based on the standard (CCME, 2000) equation provided below:

$$SQC_{SI} = \frac{(TDI - EDI) \cdot SAF \cdot BW}{[(AF_I \cdot IR)] \cdot ET} \quad + \quad [BSC]$$

Where:

SQC_{SI} = human health soil ingestion guideline (mg/kg);

TDI = tolerable daily intake for barium (0.07 mg/kg-bw/day; see Section 5.4);

EDI = estimated daily intake (0.014 mg/kg-bw/day; see Section 5.5);

SAF = soil allocation factor (0.25; see Section 8.1.1 below);

BW = body weight (16.5 kg: toddler (agricultural, residential/parkland and commercial land uses); 70.7 kg: adult (industrial land use); CCME, 2000);

AF_I = absorption (bioavailability) factor for gut (conservatively assumed to be 1.0; see Section 8.1.2 below);

IR = soil ingestion rate (0.00008 kg/day: toddler (agricultural, residential/parkland and commercial land uses); 0.00002 kg/day: adult (industrial land use); CCME, 2000);

ET = exposure term (1.0: agricultural, and residential/parkland land uses; 0.2747: commercial and industrial land uses; CCME, 2000); and,

BSC = background soil concentration (325 mg/kg; see Section 2.4.3).

Substituting the appropriate values for each land use in the above equation, and rounding to 2 significant figures, yields values of 3,200 mg/kg (agricultural and residential/parkland), 11,000 (commercial), and 180,000 mg/kg (industrial). These values are the soil quality guidelines for the human soil ingestion pathway (Table 6).

The rationale for two parameters is discussed in the following Sections.

8.1.1 Soil Allocation Factor

The CCME (1996) protocol indicates that humans are potentially exposed to environmental contaminants through one or more of 5 media: soil, water, air, food, and consumer products. Accordingly, by default, a soil allocation factor (SAF) of 0.2 is used in calculating generic guidelines for human health pathways to ensure that the total exposure from all media does not exceed acceptable limits. However, CCME (2000) allows the SAF to be calculated by only considering environmental media through which significant exposure is likely to occur.

In the case of barium, significant exposure through air is unlikely. Health Canada (1990) states that "at the levels of barium measured in U.S. air, intake of barium through inhalation would be negligible compared with the amount ingested." This leaves four environmental media through which exposure is considered possible, and accordingly an SAF of 0.25 was used in this project.

8.1.2 Human Bioavailability

It is likely that the bioavailability of barium sulphate to humans is significantly lower than that of soluble barium compounds. Certainly, repeated high doses of barium sulphate administered as a contrast medium during medical x-ray procedures generally result in no adverse affect. However, one study (McCauley and Washington, 1983) indicates that, at low doses, the uptake of barium from barium sulphate in rats may be similar to that from barium chloride (barium chloride is soluble). Hence, in the absence of any further work specifically addressing the mammalian bioavailability and/or the subchronic or chronic toxicity of barium sulphate, a mammalian bioavailability of 1.0 was assumed for barium sulphate.

8.2 Inhalation of Indoor Air Check

Barite is non-volatile, and accordingly this check is not relevant and is not calculated.

8.3 Off-Site Migration Check

The off-site migration check is calculated to ensure that wind and water erosion of contaminated material from an industrial site could not cause unacceptable contaminant concentrations on an adjacent residential property (CCME, 1996). The check is calculated using the equation provided in CCME (1999):

$$C_i = \frac{\{[D_m \cdot C_m] - [(D_m - D_d) \cdot BSC]\}}{D_d}$$

Where:

C_i = off-site migration check (mg/kg);
D_m = mixing depth (2 cm, CCME, 1999);
C_m = soil quality guideline for residential/parkland use (3,200 mg/kg, see Table 6);
D_d = depth of deposited material before mixing (0.14 cm; CCME, 1999); and,
BSC = background concentration of the contaminant in the receiving soil (325 mg/kg, Section 2.4.3).

Substituting these values in the above equation, and rounding to 2 significant figures, yields a value of 41,000 mg/kg, which is the human health off-site migration check (Table 6).

8.4 Groundwater Check (Drinking Water)

This check was not calculated because it is not applicable to metals (CCME, 1999).

8.5 Produce, Meat and Milk Check

This check applies only to non-polar organic compounds, and is not calculated for metal contaminants (CCME, 1999).

8.6 Data Gaps

The major data gap in the derivation of the soil quality guideline for barite is the lack of a high quality, subchronic or, preferably, chronic toxicological study of the effects of barite on a mammalian laboratory test species (*e.g.*, rat). In the absence of such a study, it was necessary to make the (likely very conservative) assumption that the tolerable daily intake (reference dose) developed by the USEPA for soluble barium compounds was applicable to barite.

9. APPLICATION OF BARITE SOIL QUALITY GUIDELINES

9.1 Analytical Definitions

In order to apply the soil quality guidelines for barite correctly, it is necessary to understand two analytical definitions.

9.1.1 Extractable Barium

In this document, "extractable barium" refers to the results of soils analyses conducted using the method described in Section 6.2.2. Extractable barium is the barium that is present in soil pore water plus the barium that is sorbed to clay surfaces. Extractable barium has been shown to correlate with the barium that is bioavailable to plants and soil invertebrates.

9.1.2 Total Barium

In this document, "total barium" refers to the barium results of soils analyses conducted using one of the "standard acid" methods (see Section 2.2) for measuring environmental concentrations in soils. These methods use an acid digest (HNO_3, or HNO_3/HCl, but not HF) typically followed by inductively coupled plasma – optical emission spectrometry (ICP-OES) detection. Total barium analysis measures a part of the barium bound in mineral particles in addition to that measured by the extractable barium technique. The guidelines for Barite Sites (see below) are expressed in terms of total barium concentrations.

9.2 Guideline Application (Figure 1)

At sites where barium contamination is suspected, the assessor may first measure total barium and compare to the CCME (1999) barium guidelines (Row C of Table 6). Since these are the most stringent guideline values, no further action will be necessary if soil barium concentrations fall below the applicable CCME guideline.

Where the assessor suspects or has confirmed that barium concentrations in soil exceed the CCME (1999) guidelines, the following procedure should be used. This procedure is based on using the guideline for extractable barium to determine whether the Alberta barite guidelines can be used at a particular site. The steps for applying the barite soil quality guidelines are as follows:

1. During initial site investigation, if barite contamination is suspected, sufficient samples should be analyzed for extractable barium to allow a determination of whether or not extractable barium at the site exceeds the applicable guideline in Row A of Table 6.
2. If all extractable barium samples are below the applicable guideline, then the site is deemed to be a "Barite Site" and the guidelines in Row B of Table 6 can be applied to total barium concentrations at the site.
3. If all extractable barium samples are not below the applicable guideline in Row A of Table 6, then the site is not a "Barite Site" and the guidelines in Row B of Table 6 cannot be applied to total barium concentrations at the site. Instead, a proponent must either use the CCME (1999) guidelines for barium (Row C in Table 6) to assess the contamination, or conduct a site-specific risk assessment.

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TABLES

Table 1. Physical and Chemical Properties of Barite

Property	*Value*	*Units*	*Source*
CAS number	7727-43-7	na	ChemFinder (2003)
Molecular formula	$BaSO_4$	na	CEPA (2002)
Mineral class	sulphate	na	Mineral Galleries (2003)
Physical State	crystals or powder	na	CEPA (2002)
Molecular weight	233.3876	g	ChemFinder (2003)
Hardness	3 to 3.5	Mohs scale	Nova Scotia Natural Resources (1992)
Specific gravity	4.2 to 4.6	na	ADMMR (2001)
Density	4.5 at 15 °C	g/cm^3	WHO (1996)
Melting point	1,580	°C	ChemFinder (2003); USEPA (1999)
Boiling point	1,149	°C	USEPA (1999)
Vapour pressure	"no data"	na	USEPA (1999)
Solubility Product Constant (K_{sp} at 25 °C)	1.1×10^{-10}	na	ACC (2003)
Water solubility	1.15 at 0 °C	mg/L	USEPA (1999)
Water solubility	2 at 18 °C	mg/L	HSBD (2002) cited in CEPA (2002)
Water solubility	2.4 at 25 °C	mg/L	USEPA (1997)
Water solubility	2.85 at 30 °C	mg/L	WHO (1996)
Organic solvent solubility	insoluble	mg/L	CEPA (2002)

Notes.

na = not applicable

Table 2. Selected Plant Toxicological Data for Barite

Organism	*Effect (% decrease)*	*Endpoint*	*Concentration (mg barite / kg dry soil)*	*Soil pH*	*Test Substrate*	*Reference*
Alsike Clover	**Emergence (32%)**	LOEC	1,000,000	8 3 – 9 1	Clay loam; 1 2% organic matter	ESG (2003a)
(*Trifolium hybridum*)	**Shoot length (20%)**	LOEC	1,000,000	8 3 – 9 1		
	Shoot wet mass	NOEC	1,000,000	8 3 – 9 1		
	Shoot dry mass	NOEC	1,000,000	8 3 – 9 1		
	Root length	NOEC	1,000,000	8 3 – 9 1		
	Root wet mass	NOEC	1,000,000	8 3 – 9 1		
	Root dry mass	NOEC	1,000,000	8 3 – 9 1		
	Nodule abundance	NOEC	1,000,000	8 3 – 9 1		
Orchardgrass	Emergence	NOEC	1,000,000	8 3 – 8 6	Clay loam; 1 2% organic matter	ESG (2003a)
(*Dactylis glomerata*)	Shoot length	NOEC	1,000,000	8 3 – 8 6		
	Shoot wet mass	NOEC	1,000,000	8 3 – 8 6		
	Shoot dry mass	NOEC	1,000,000	8 3 – 8 6		
	Root wet mass	NOEC	1,000,000	8 3 – 8 6		
	Root dry mass	NOEC	1,000,000	8 3 – 8 6		
	Nodule abundance	NOEC	1,000,000	8 3 – 8 6		
	Root length	IC20	850,000	8 3 – 8 6		

Table 2. Selected Plant Toxicological Data for Barite (Cont.)

Organism	***Effect (% decrease)***	***Endpoint***	***Concentration (mg barite / kg dry soil)***	***Soil pH***	***Test Substrate***	***Reference***
Perennial ryegrass (*Lolium perenne*)	Emergence	NOEC	1,000,000	8 3 – 8 7	Clay loam; 1 2% organic matter	ESG (2003a)
	Shoot length	NOEC	1,000,000	8 3 – 8 7		
	Shoot wet mass	NOEC	1,000,000	8 3 – 8 7		
	Shoot dry mass	NOEC	1,000,000	8 3 – 8 7		
	Root wet mass	NOEC	1,000,000	8 3 – 8 7		
	Root dry mass	NOEC	1,000,000	8 3 – 8 7		
	Nodule abundance	NOEC	1,000,000	8 3 – 8 7		
	Root length (23%)	LOEC	1,000,000	8 3 – 8 7		
	Root length	IC20	960,000	8 3 – 8 7		
Green beans (*Phaseolus vulgaris* L , 'Tendergreen')	Plant dry mass	NOEC	227,500	6 2	Dagor silt loam; 8 1% organic matter	Miller *et al* (1980)
	Plant dry mass	NOEC	795,300	6 2		
Sweet corn (*Zea mays* var saccharata (Sturtev) 'Bailey' Northrup King 199)	Plant dry mass	NOEC	227,500	6 2	Dagor silt loam; 8 1% organic matter	Miller *et al* (1980)
	Plant dry mass (20%)	LOEC	795,300	6 2		

Table 3. Selected Invertebrate Toxicological Data for Barite

Organism	*Effect*	*Endpoint*	*Concentration (mg barite / kg dry soil)*	*Soil pH*	*Test Substrate*	*Reference*
Earthworm (*Eisenia fetida*)	Survival	NOEC	17,000	5.0	Artificial soil, 11% clay, 18% silt, 71% sand; 1.2% organic matter	Simini *et al.* (2002)
Enchytraeid worm (*Enchytraeus crypticus*)	Survival	NOEC	17,000	5.0	Artificial soil; 1.2% organic matter	Kuperman *et al.* (2002)
Earthworm (*Eisenia andrei*)	Survival	NOEC	1,000,000	8.4 – 8.5	Clay loam; 1.2% organic matter	ESG (2003a)
Springtail (*Onychiurus folsomi*)	Survival	NOEC	1,000,000	8.2 – 8.4	Clay loam; 1.2% organic matter	ESG (2003a)

Table 4. Acute Barite Toxicological Data for Mammals

Organism	*Effect*	*Endpoint*	*One Time Dose (mg/kg body weight)*	*Exposure Period*	*Exposure Route*	*Reference*
CBL-Wistar albino rats	Mortality	NOEL	160,000	Acute – 1 time	Intragastric cannula	Boyd and Abel (1966)
	Mortality (from stomach rupture)	LD_{50}	307,000	Acute – 1 time	Intragastric cannula	
	Mortality (from bowel obstruction)	LD_{0}	163,000	Acute – 1 time	Intragastric cannula	
	Mortality (from bowel obstruction)	LD_{50}	364,000	Acute – 1 time	Intragastric cannula	
	Mortality (from bowel obstruction)	LD_{100}	564,000	Acute – 1 time	Intragastric cannula	

Table 5. Chronic Barite Toxicological Data for Mammals

Organism	Effect	Endpoint	Exposure Concentrations (mg/kg food)	Exposure Period	Exposure Route	Estimated Dose (mg/kg bw per day)	Reference
CF-1 mice	General appearance	NOEL	8 (maximum concentration)	3 generations	oral	1.067 [1]	Hutcheson *et al* (1975) [2]
	Morbidity and mortality	NOEL	8 (maximum concentration)	3 generations	oral	1.067 [1]	
	Abnormalities at necropsy	NOEL	0.8 (maximum concentration)	3 generations	oral	0.107 [1]	
	Weight gain	NOEL	8 (maximum concentration)	3 generations	oral	1.067 [1]	
	Reproduction and lactation (number of females having litters, average litter size, average weanling weight)	NOEL	8 (maximum concentration)	3 generations	oral	1.067 [1]	
	Hematology and serum protein levels	NOEL	0.8 (maximum concentration)	3 generations	oral	0.107 [1]	

Notes.

1. *Calculated from food concentration using a value of 0.03 kg for body mass (Health Canada, 1994) and an ingestion rate of 4 g/day (Health Canada, 1994)*
2. *Hutcheson et al. (1975) fed mice food containing barite and oxides of scandium, chromium, lanthanum, samarium, europium, terbium, dysprosium, thulium, and ytterbium.*

Table 6. Soil Quality Guidelines for Barite

THE PROCEDURE FOR APPLYING SOIL QUALITY GUIDELINES FOR BARITE:IS FOUND IN SECTION 9.2 and FIGURE 1.

	LAND USE				
	Natural Area ***(mg/kg)***	***Agricultural*** ***(mg/kg)***	***Residential/ Parkland*** ***(mg/kg)***	***Commercial*** ***(mg/kg)***	***Industrial*** ***(mg/kg)***
A: Guideline for Extractable Barium	***260***	***260***	***260***	***440***	***440***
B: Guideline for Total Barium at Barite Sites	**3,300**	**3,200**	**3,200**	**11,000**	**41,000**
Human Health					
SQG_{HH}	—	3,200	3,200	11,000	485,000
Soil ingestion guideline	—	3,200	3,200	11,000	180,000
Inhalation of indoor air check	—	NC [a]	NC [a]	NC [a]	NC [a]
Off-Site migration check	—	—	—	—	41,000
Groundwater check (drinking water)	NC [b]	NC [b]	NC [b]	NC [b]	NC [b]
Produce, meat, and milk check	—	NC [c]	NC [c]	NC [c]	NC [c]
Environment					
SQG_E	3,300	3,300	180,000	180,000	180,000
Soil contact guideline	180,000	180,000	180,000	180,000	180,000
Soil ingestion guideline	3,300	3,300	—	—	—
Nutrient and energy cycling check	NC [d]	NC [d]	NC [d]	NC [d]	NC [d]
Off-site migration check	—	—	—	—	NA
Groundwater check (aquatic life)	NC [b]	NC [b]	NC [b]	NC [b]	NC [b]
C: CCME (1999) Guideline for Total Barium at Non-Barite Sites	---	**750**	**500**	**2,000**	**2,000**

Notes:

--- = guideline/check value is not part of the exposure scenario for this land use and therefore is not calculated.

NC = not calculated.

NA = not applicable: pathway not part of the exposure scenario for this land use or calculated guideline value exceeds 1,000,000 mg/kg

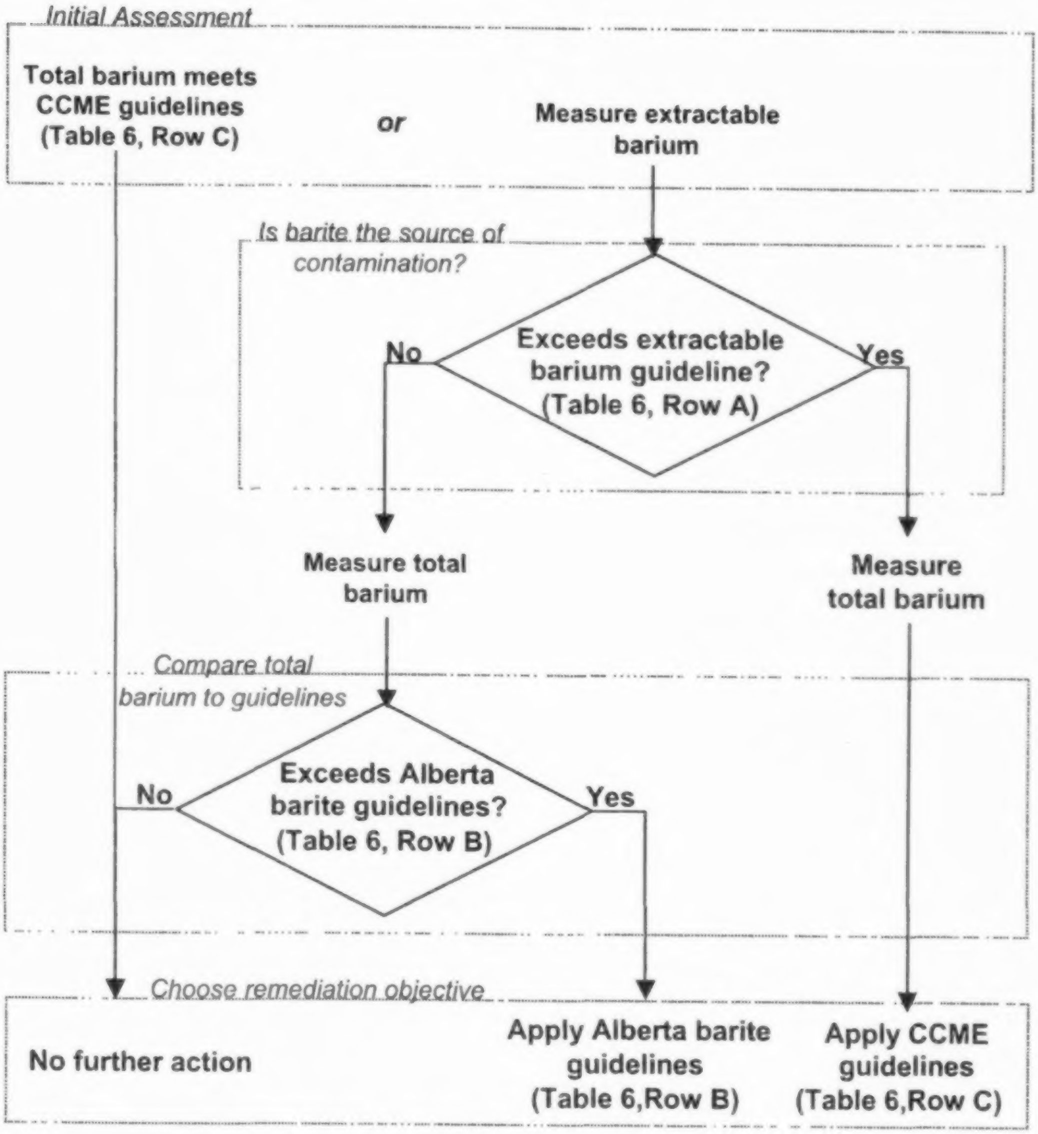
a This guideline is not calculated because barite is non-volatile.

b. Applies to organic compounds and is not calculated for metal contaminants.

c The produce, milk and meat check applies to organic contaminants and is thus not calculated for inorganic contaminants.

d. Data are insufficient/inadequate to calculate these guidelines/check values.

Figure 1. Guideline Application Procedure


Initial Assessment
Total barium meets CCME guidelines (Table 6, Row C)
or
Measure extractable barium
Is barite the source of contamination?
Exceeds extractable barium guideline? (Table 6, Row A)
No
Yes
Measure total barium
Measure total barium
Compare total barium to guidelines
Exceeds Alberta barite guidelines? (Table 6, Row B)
No
Yes
Choose remediation objective
No further action
Apply Alberta barite guidelines (Table 6,Row B)
Apply CCME guidelines (Table 6,Row C)

TECHNICAL APPENDICES FOR BARITE SOIL QUALITY GUIDELINES

Alberta Environment

TECHNICAL APPENDICES FOR

BARITE SOIL QUALITY GUIDELINES

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for:

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and

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February 2004

Pub. No: T/739
ISBN No. 0-7785-3165-1 (Printed Edition)
ISBN No. 0-7785-3166-X (On-line Edition)
Web Site: http://www.gov.ab.ca/env/info/infocentre/publist.cfm

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EXECUTIVE SUMMARY

Barite (barium sulfate) serves as a weighting agent in oilfield drilling muds and it commonly remains onsite after drilling operations are completed. The work described in this report is part of a project that was undertaken to develop soil quality guidelines for barite. Current guidelines for barium in soil appear to be based on background levels and/or the properties of soluble barium compounds. Under typical environmental conditions, barite has a low aqueous solubility and is thought to have a low bioavailability, and accordingly, soil quality guidelines for barite are expected to be less stringent than those for soluble barium.

Information in the literature has clearly demonstrated that under sulfate-reducing conditions, microbial processes will consume sulfate and thereby increase the concentrations of dissolved Ba^{++} in cultures or environments that contain barite. However, there is little or no information on the dissolution of barite by microbial activities under other redox conditions. Thus, the objective of this study was to determine whether the concentration of dissolved Ba^{++} increased in barite-containing microcosms that were subjected to various redox conditions that promoted different microbial processes. Barite was added to microcosms containing uncontaminated (background) soil, appropriate liquid media and an electron donor (either glucose or lactate). Different sets of microcosms were incubated at room temperature under aerobic, nitrate-reducing, Mn(IV)-reducing, Fe(III)-reducing, sulfate-reducing and methanogenic conditions. The amounts of soluble Ba^{++} were determined over a 4-month incubation period.

The release of soluble Ba^{++} was observed under aerobic, Mn(IV)-reducing, Fe(III)-reducing and sulfate-reducing conditions. The findings of this short-term investigation, using microcosms with ample supplies an electron donor suggest that there is a potential for barite dissolution releasing soluble Ba^{++} in soil environments that are dominated by aerobic, Mn(IV)-reducing, Fe(III)-reducing or sulfate-reducing conditions. The rates of microbial release of soluble Ba^{++} in these types of environments would likely be much slower than observed in this study, because the supply of electron donor would be rate limiting in the environment.

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1 INTRODUCTION

Barite (barium sulfate) serves as a weighting agent in oilfield drilling muds and it commonly remains onsite after drilling operations are completed. The work described in this report is part of a project that was undertaken to develop soil quality guidelines for barite. Current guidelines for barium in soil appear to be based on background levels and/or the properties of soluble barium compounds. Under typical environmental conditions, barite has a low aqueous solubility and is thought to have a low bioavailability, and accordingly, soil quality guidelines for barite are expected to be less stringent than those for soluble barium.

The toxicity of a barium salt is highly dependent on the solubility of the salt. For example, barium sulfate is extremely insoluble and it is routinely administered orally to facilitate x-ray imaging for the detection of gastric ulcers. Barium sulfate is not absorbed from the intestinal lumen in patients that are administered this suspension (Jourdan et al. 2001). In contrast, barium ions from soluble salts are very toxic, and ingestion of aqueous barium chloride has been used as a means of suicide (Jourdan et al. 2001). The antidote for ingested soluble barium is a soluble form of sulfate, which precipitates soluble Ba^{++} as the nontoxic barium sulfate in the stomach.

Microbial dissolution of barium sulfate has been reported in the literature. This typically occurs under anaerobic conditions, with sulfate-reducing bacteria being implicated or shown to be the microorganisms responsible for causing the release of soluble Ba^{++} by reducing the sulfate moiety, thereby liberating soluble Ba^{++}. The following paragraphs summarize the published literature on the topic of release of soluble Ba^{++} from barium sulfate under different laboratory and environmental conditions.

Bolze et al. (1974) incubated lake mud under anaerobic conditions in 200 mL of medium supplemented with powdered barite and H_2 as the energy source. After 17 days of incubation at 20°C, the average concentration of dissolved Ba^{++} was 3.3 mg/L in the viable cultures, whereas the average concentration in the sterile controls was 0.5 mg/L. Another set of cultures that contained barite and ferrous sulfate was also incubated and analyzed for soluble Ba^{++}. In this set, the Ba^{++} concentration was <0.1 mg/L, indicating that the dissolution of barite was suppressed by the presence of the more soluble ferrous sulfate.

Microbially-mediated release of Ba^{++} from uranium mine tailings has been investigated because of the potential simultaneous release of radioactive $^{226}Ra^{++}$. At many uranium mining and milling sites, soluble radium is co-precipitated with $BaSO_4$ yielding a $(Ba,Ra)SO_4$ precipitate that settles as a sludge that is stored in tailings ponds. Fedorak et al. (1986) analyzed two of these co-precipitates and demonstrated that on a mass basis, the sludges were predominately $BaSO_4$.

McCready et al. (1980) produced a synthetic, $(Ba,Ra)SO_4$ precipitate in which the Ba, Ra, and S were all radioactive atoms. They added this to a pure culture of the sulfate-reducing bacterium, *Desulfovibrio vulgaris*, in medium that contained lactate as the energy source and dissolved sulfate along with the insoluble sulfate from the $(Ba,Ra)SO_4$. Over a 19-day incubation at 25°C, the concentration of soluble Ba^{++} increased at nearly a linear rate to a maximum concentration of 5.4 mg/L. This was only about 3% of the concentration expected based on the amount of sulfide released from sulfate reduction. During the growth of the *D. vulgaris*, CO_2 was produced leading to the re-precipitation of much of the Ba^{++} released from $(Ba,Ra)SO_4$ as $BaCO_3$ (McCready et al. 1980).

$(Ba,Ra)SO_4$ sludges from two Canadian uranium mines were used in laboratory studies to evaluate the release of $^{226}Ra^{++}$ and Ba^{++} by indigenous microorganisms (Fedorak et al. 1986). Prior to use in the microbiological studies, these sludges were washed free of dissolved sulfate. Microbial counts showed that the samples contained $>10^4$ sulfate-reducing bacteria/mL, and nutrient supplementation experiments demonstrated that each of the five energy sources tested (glucose, lactic, pyruvic, acetic, and glycollic acids) stimulated the microbial reduction of sulfate. In general increasing concentrations of lactate increased the amounts of Ba^{++}, and $^{226}Ra^{++}$, released from the the sludges by the end of 8- to 10-week incubation periods. In one experiment, nitrate was added as a nitrogen source for the microorganisms in one of the $(Ba,Ra)SO_4$ sludges. In the presence of nitrate, gas evolution from denitrification was evident, and the release of $^{226}Ra^{++}$ was only 3% of that in the absence of nitrate (Fedorak et al. 1986). This observation suggests that nitrate-reducing conditions inhibited the release of $^{226}Ra^{++}$. Thus, it is likely that the release of Ba^{++} would also have been inhibited under nitrate-reducing conditions, although the soluble Ba^{++} concentrations were not measured during this experiment.

Martin et al. (2003) studied the release of $^{226}Ra^{++}$ from $(Ba,Ra)SO_4$ sediments in a disposal pond at a Canadian uranium mine. They analyzed porewaters taken from various depths and three different locations in the pond. One of the locations had thick vegetative growth (*Chara* sp.) whereas the other two locations had sparse vegetative growth. Among the parameters measured were dissolved oxygen and soluble iron, manganese, barium and ^{226}Ra. The dissolved oxygen concentrations dropped sharply in the sediments and these became anaerobic within a few millimeters of the sediment-water interface. Although dissolved $^{226}Ra^{++}$ and Ba^{++} were observed in most of the porewater samples, the highest concentrations were found in the location with the thick vegetation. There was a strong linear correlation between the concentrations of the dissolved $^{226}Ra^{++}$ and Ba^{++}. At two locations, elevated concentrations of dissolved iron and manganese were observed at depths where increased concentrations of dissolved $^{226}Ra^{++}$ and Ba^{++} occurred. Plant growth would provide organic carbon for the microbial community in the sediment and the overlaying water, thereby driving processes such as Fe(III) reduction, Mn(IV) reduction and sulfate reduction. These microbial processes would play a major role in the dissolution of Fe(II), Mn(II), $^{226}Ra^{++}$ and Ba^{++}.

Scale and sludge deposits formed during oil production can contain ^{226}Ra in the form of $(Ba,Ra)SO_4$. The activities of sulfate-reducing bacteria have the potential to release $^{226}Ra^{++}$ from this naturally occurring radioactive material (NORM), in the same way they release $^{226}Ra^{++}$ from uranium tailings. Phillips et al. (2001) isolated a sulfated-reducing bacterium, designated *Desulfovibrio* sp. strain AZK1, from an oil-field brine pond and tested its ability to release $^{226}Ra^{++}$ and Ba^{++} from oil-field materials containing barite as the major form of sulfate. Cultures were supplemented with lactate and incubated at 30°C. The greatest increase in dissolved Ba^{++} occurred during the first day of incubation while the dissolved sulfate was depleted from the medium. Sulfate consumption was accompanied by sulfide formation, but the release of soluble Ba^{++} was not stoichiometric with the sulfide production. In fact, the molar ratios of dissolved Ba^{++} to sulfide production was <0.002. Although the reason for the extremely low molar ratio could not be determined, Phillips et al. (2001) concluded that none of the following mechanisms was a major contributor to the low ratio: precipitation as BaS or $BaCO_3$, adsorption by cells or reactions with reduced cell products.

Baldi et al. (1996) described transformations of barium in sewage sludges from treatment plants near Florence, Italy. The major source of barium at one of the plants was from hospital x-ray wastes. These investigators also observed lower than expected soluble Ba^{++} concentrations based on the amount of sulfide that was produced. They attributed this to the formation of $BaCO_3$ and the transient appearance of BaS. Aeration of an anaerobic sewage sludge sample caused the redox potential to increase, and brought about an inexplicable decrease in the total barium in the sludge. Baldi et al. (1996) presented several possible explanations for this loss of barium, but none was very convincing.

Sulfate reduction occurs in the contaminated groundwater of the Norman Landfill in Oklahoma (Ulrich et al. 2003). Barite was found to be an important source of sulfate for this process at intermediate aquifer depths (Ulrich et al. 2003). In general, there was an inverse relationship between the groundwater concentrations of sulfate and barium. That is, as sulfate was depleted with depth, the dissolved Ba^{++} concentrations tended to increase. Upgradient from the landfill, the concentrations of dissolved organic carbon, sulfate and barium were 2.9 mg/L, 1.2 mM (approximately 120 mg/L), and 1 µM (approximately 140 µg/L), respectively. Downgradient from the landfill and within the anoxic plume, these concentrations were 159 mg/L, 0.1 mM (approximately 10 mg/L), and 52 µM (approximately 7,100 µg/L), respectively. These measurements suggest that sulfate reduction was releasing Ba^{++} from barite in the sediments in the aquifer. Interestingly, the Fe^{++} concentration also increased, with the highest concentration being 145 times greater in the landfill leachate-contaminated groundwater than in the background sample of groundwater. The dissolved Ba^{++} and Fe^{++} were presumed to have originated from constituents of the alluvium.

Ulrich et al. (2003), also performed laboratory studies to verify that Ba^{++} release was due to the activities of sulfate-reducing bacteria. The sediment slurries incubated in the laboratory clearly demonstrated that sulfate consumption led to soluble Ba^{++} release from naturally occurring barite and from supplemented barium sulfate. Furthermore, some cultures were treated with molybdate (5 mM), which is a potent inhibitory of sulfate-reducing bacteria. No release of soluble Ba^{++} was observed in those cultures.

2 OBJECTIVES

It is has been clearly demonstrated that under sulfate-reducing conditions, microbial processes will consume sulfate and thereby increase the concentrations of dissolved Ba^{++} in cultures or environments that contain barite (e.g. McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). However, it was hypothesized that microbial activities would not increase the concentration of dissolved Ba^{++} from barite under other redox conditions including: aerobic, nitrate-reducing, Mn(IV)-reducing, Fe(III)-reducing and methanogenic conditions. Thus, the objective of this study was to determine whether the concentration of dissolved Ba^{++} increased in barite-containing microcosms that were subjected to these various redox conditions that promote different microbial processes.

3 MATERIALS AND METHODS

3.1 Soil samples

Two soil samples were provided by Komex International Ltd. One sample, designated as the contaminated soil, contained drilling mud that had been spilled in late 1985. The other soil sample, referred to as the background soil, contained no drilling mud. Both soils were collected from an oilfield lease in the vicinity of Pincher Creek, Alberta. The contaminated soil was collected from a stockpile of excavated material. The material was excavated as part of a process to remediate soil at the site contaminated with invert drilling mud (contaminants including weathered diesel and barium sulfate). The background sample was collected from subsoil in an uncontaminated part of the same lease site. Top soil was removed, and the sample was collected from a depth of approximately 1 m below ground surface.

Analyses done by Enviro-Test Laboratories showed that the barium concentrations in the background and the contaminated soils were 204 and 2,150 mg/kg, respectively. Enumerations of sulfate-reducing bacteria (done in our laboratory) gave numbers of 9/g and 9.3 x 10^3/g (wet weight) in the background and contaminated soils, respectively.

3.2 Microcosm preparation

One hundred and twelve microcosms were established for this study. They are summarized in Table 3.1, and described in more detail in the following sub-sections based on the terminal electron acceptor process that was studied.

The aerobic microcosms, in which O_2 was the terminal electron acceptor, were incubated in Erlenmeyer flasks with foam plugs to allow air exchange into and out of the flasks. All of the other microcosms were prepared using strict anaerobic techniques to ensure that O_2 was excluded. Each microcosm received 50 g of soil as the source of microorganisms. For the anaerobic microcosms, the soil was dispensed into sterile 158-mL serum bottles in an anaerobic chamber with an atmosphere of 5% H_2, 10% CO_2, balance N_2. Although H_2 serves as an electron donor (energy source) in most anaerobic environments, no attempt was made to remove the H_2 from the headspace gas of the serum-bottle microcosms.

To prepare the sterile controls, covered flasks or sealed serum bottles containing the 50-g soil sample were autoclaved for 1 h on 3 consecutive days. After adding the sterile medium to the autoclaved soil, the controls were supplemented with chloroform to a final concentration of 1% (v/v) to further reduce the chances of microbial growth (Albrechtsen et al. 1995).

Table 3.1 Summary of the microcosms established during this study.

Condition	Incubation in	No. of sterile controls	No. of viable cultures with BaSO$_4$	No. of viable cultures without BaSO$_4$
Aerobic	Shake flasks	2	5[e]	2[e]
Nitrate-reducing	Serum bottles	2	5[e]	2[e]
Mn(IV)-reducing	Serum bottles	2	5[e]	2[e]
Fe(III)-reducing	Serum bottles	2	5[e]	2[e]
Sulfate-reducing	Serum bottles	2	5[e] + 5[c]	2[e]
Sulfate-reducing[d]	Serum bottles	2[d]	5[d,e] + 5[d,f]	2[d,e] + 2[d,f]
Sulfate-reducing[g]	Serum bottles	2[g]	5[g,c] + 5[g,f]	2[g c] + 2[g,f]
Sulfate-reducing[g,h]	Serum bottles	2[g,h]	5[g,h,c] + 5[g,h,f]	0
Methanogenic-AS[a]	Serum bottles	2	5[c]	2[e]
Methanogenic-NAS[b]	Serum bottles	2	5[e]	2[e]

[a] Supplemented with anaerobic sewage sludge as a source of methanogenic community

[b] No anaerobic sewage sludge supplementation.

[c] Supplemented with 1 g lactate/L (100 mg lactate/microcosm).

[d] Contaminated soil

[e] Supplemented with 1 g glucose/L (100 mg glucose/microcosm).

[f] Without electron donor supplementation

[g] Background soil supplemented with 10 mL SRB enrichment culture.

[h] Each microcosm contains 10,000 ppm (500 mg) of barium sulfate

For each incubation condition, two sterile controls, five or ten viable microcosms supplemented with barium sulfate, and two or four microcosms without barium sulfate were prepared (Table 3.1). High purity (98%) barium sulfate (Aldrich Chemical Company, Milwaukee, WI) was used in these experiments. In most cases, 100 mg of barium sulfate was added to the microcosms (Table 3.1), which was equivalent to a soil that contained 2,000 ppm barium sulfate. In a few cases, 500 mg of barium sulfate was added to the microcosms (Table 3.1), which was equivalent to a soil that contained 10,000 ppm barium sulfate. The appropriate amount of barium sulfate was added to the soil-containing serum bottle just before the sterile culture medium was added. The microcosms were then sealed and in most cases the viable microcosms were supplemented with an electron donor.

The media used by Fedorak et al. (1997) were modified for use in these microcosms studies. In each case, sulfate was omitted from the medium so that the only source of sulfate was the sulfate in the soil or the added barium sulfate. Sulfate-free media were used to prevent re-precipitation of any Ba^{++} that may have been released in the microcosms. Each medium contained an appropriate electron acceptor, and in the case of the sulfate-reducing microcosms, barium sulfate served as the sole sulfate source. To ensure that there would be microbial activity in the viable microcosms, suitable electron donors were added (Table 3.1). In most cases, glucose was added to the microcosms. However, lactate was added to some of the sulfate-reducing microcosms. After the glucose was depleted from the medium more glucose or lactate was added to the appropriate microcosms.

All of the microcosms were incubated in the dark at room temperature (approximately 22°C). From each group of five viable microcosms with barium sulfate (column 4, Table 3.1), only three microcosms were monitored for microbial activity. The other two microcosms were established as "spares" to be monitored if one of the other three microcosms was inadvertently damaged.

3.2.1 Aerobic microcosms

Each aerobic microcosm contained 50 g of background soil in a 500-mL Erlenmeyer with 100 mL of medium (see Appendix, section A1). Nine microcosms were prepared. Five of these contained viable cultures, with 100 mg of barite added, and were supplemented with 1 mL of a 100 g glucose/mL solution for a final glucose concentration of 1 g/L. Two aerobic microcosms contained viable cultures, but no barite was added. These were also supplemented glucose. Glucose was replenished in these seven microcosms on a weekly basis.

The last two microcosms were sterile controls. Each was prepared by adding 5 mL of water to 50 g of soil for and this was autoclaved for 1 h on 3 consecutive days. Sterile medium (100 mL) and 100 mg of barite were added to each flask along with chloroform to a final concentration of 1% v/v to minimize chances of microbial growth. No glucose was added to the sterile controls.

All of the microcosms were incubated in the dark at room temperature, shaking at 200 rpm. The two sterile controls, two viable microcosms with no barite, and three of the viable microcosms with barite were monitored for soluble Ba^{++}, and glucose. At each sampling time, about 5 mL of slurry were removed from these seven microcosms using a sterile wide-mouth, 10-mL pipette. The other two viable microcosms with barite were not monitored but kept as spares in case something happened to one of the other three microcosms.

3.2.2 Nitrate-reducing microcosms

The medium composition and method of preparation of the medium used for the nitrate-reducing microcosms are given in section A.3 of the Appendix. Only the background soil was used as a source of microorganisms in these microcosms. While in the anaerobic chamber, each soil-containing serum bottle received 100 mL of nitrate-reducing medium and 1 mL of a sterile, anoxic solution that contained 100 g glucose/mL.

3.2.3 Mn(IV)-reducing microcosms

Section A.4 in the Appendix summarizes the composition and preparation of the medium used in the Mn(IV)-reducing medium. One hundred millilitres of this medium containing a slurry of MnO_2 as the source of Mn(IV) were added to each serum bottle of background soil along with 1 mL of anoxic, sterile glucose solution.

3.2.4 Fe(III)-reducing microcosms

The medium composition and method of preparation of the medium used for the Fe(III)-reducing microcosms are given in section A.4 of the Appendix. Fe(III) was added as a slurry of $Fe(OH)_3$. Only the background soil was used as a source of microorganisms in these microcosms. In the anaerobic chamber, each soil-containing serum bottle received 100 mL of Fe(III)-reducing medium and 1 mL of a sterile, anoxic glucose solution (100 g/mL).

3.2.5 Sulfate-reducing microcosms

Sulfate-reducing microcosms were established using four different conditions (Table 3.1). These were: (1) using background soil as the source of microorganisms, and adding 100 mg of barium sulfate; (2) using contaminated soil as the source of microorganisms, and adding 100 mg of barium sulfate; (3) using background soil supplemented with a mixed culture of sulfate-reducing bacteria, and adding 100 mg of barium sulfate; and (4) using background soil supplemented with a mixed culture of sulfate-reducing bacteria, and adding 500 mg of barium sulfate. In most cases, glucose was added as the electron donor. However, few sulfate-reducing bacteria use glucose and it is typically metabolized by other microorganisms leaving products that can be used by sulfate-reducing bacteria. Thus, the addition of glucose indirectly stimulates the activities of sulfate-reducing bacteria. Some microcosms were supplemented with lactate (Table 3.1) which is used directly by many sulfate-reducing bacteria.

Section A.5 in the Appendix summarizes the composition of the medium used in the sulfate-reducing microcosms. One hundred millilitres of this sulfate-free medium were added to each serum bottle containing the appropriate soil inoculum along with 1 mL of anoxic, sterile glucose or lactate solution. Sulfate in the soil and the added barium sulfate served as the electron acceptor in these microcosms.

The numbers of sulfate-reducing bacteria in each soil sample were determined using a three-tube most probable number method (section 3.4). Because of the low numbers of sulfate-reducing bacteria in the background soil (9/g wet weight), a mixed culture of sulfate-reducing bacteria was added to some of the microcosms that received the background soil to help reduce the time to obtain active sulfate reduction in the barium sulfate-containing microcosms. This mixed culture was derived from the contaminated soil as follows. After incubating for 1 month, there was abundant growth of sulfate-reducing bacteria in each 10^{-1} dilution tube of medium in the most probable number procedure. The contents of each of these three tubes were transferred to separate serum bottles that contained 100 mL of modified Butlin's medium (Fedorak et al. 1987). These three serum-bottle cultures were incubated for 6 weeks and then the cells were collected and washed to remove residual sulfate from the modified Butlin's medium, as outlined below.

The three 100-mL cultures were shaken vigorously and equal volumes, of about 150 mL, were transferred into each of two sterile centrifuge bottles that were being flushed with O_2-free nitrogen. These were centrifuged at 16,000 x *g* for 20 min, and the supernatant was removed. Then 75 mL of sterile, anoxic 0.85% saline (0.85 g NaCl in 100 mL water) was added to each centrifuge bottle, under a stream of O_2-free nitrogen, and the cells were re-suspended. Eleven milliliters of cell suspension from each bottle were transferred to an O_2-free gas-flushed serum bottle. This was sealed and autoclaved to provide heat-killed cells for the control microcosms.

The remainder of the suspension (about 130 mL) from each centrifuge bottle was transferred into a sterile, O_2-free gas-flushed, 300-mL centrifuge bottle containing sterile, anoxic sulfate-free medium for sulfate-reducing bacteria (section A.5). Nine-millilitre portions of this cell suspension were added to each of the 24 microcosms containing the background soil sample. The residual sulfate concentration and the numbers of sulfate-reducing bacteria in the remaining suspension were determined. The cell suspension contained 9 mg sulfate/L and 2.3×10^6 sulfate-reducing bacteria/mL.

3.2.6 Methanogenic microcosms

The medium composition and method of preparation of the medium used for the methanogenic microcosms are given in section A.6 of the Appendix. Only the background soil was used as a source of microorganisms in these microcosms. While in the anaerobic chamber, each soil-containing serum bottle received 100 mL of methanogenic medium and 1 mL of a sterile, anoxic solution that contained 100 g glucose/mL. It was not known whether the background soil would contain any methanogens, which are fastidious, strict anaerobes. Therefore two sets of methanogenic microcosms were established (Table 3.1). One set received only the soil, and a second set received the soil and 10 mL of anaerobic, domestic sewage sludge from the Gold Bar Waste Water Treatment Plant in Edmonton. This sludge has served as a source of methanogenic microorganisms in many previous studies in our laboratory.

3.3 Monitoring microbial activities in the microcosms

Table 3.2 summarizes the various microcosms that were established and the times at which samples will be taken for specific chemical analyses. At each sampling time, a 10-mL syringe was used to remove slurry samples from an anaerobic microcosm. Each sample was transferred to a 15-mL, plastic screw cap tube (Sarstedt, Newton NC). The samples were then stored at -20°C until sub-samples were removed after quickly thawing the samples. The samples were centrifuged at the highest speed of a bench-top clinical centrifuge (International Equipment Company, Needham, Mass) for 5 min to provide the supernatant for analyses.

3.4 Enumeration of sulfate-reducing bacteria

Sulfate-reducing bacteria were enumerated using a three-tube most probable number method with modified Butlin's medium in Kaput®-covered tubes (Fedorak et al. 1987). Each tube contained two acid-washed 2.5-cm finishing nails to help reduce the medium and to serve as an indicator of sulfate reduction. Sulfide produced by the sulfate-reducing bacteria reacted with the nail to yield black iron sulfide. Thus, those tubes of medium that contained a black nail after 30 days incubation at room temperature, were scored positive for growth of sulfate-reducing bacteria.

Table 3.2 Summary of the microcosms and the planned monitoring schedule for this project.

Microcosms	Sampling times	Analyses done
Aerobic	0, 7, 15, 30, and 60 days	Ba^{++}, glucose
Nitrate-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, nitrate
Mn(IV)-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, Mn(II)
Fe(III)-reducing	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, Fe(II)
Sulfate-reducing: Background and contaminated soils	0, 0.5, 1, 2, 3, and 4 months	Ba^{++}, glucose, sulfide
Sulfate-reducing: Background soil supplemented with SRB and 500 mg $BaSO_4$	0, 0.5, 1, 2, 3, and 4 months	Ba^{++}, sulfide
Methanogenic-without sewage	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, sulfide, methane
Methanogenic-with sewage	0, 0.5, 1, 3, and 4 months	Ba^{++}, glucose, sulfide, methane

3.5 Analytical methods

3.5.1 Glucose analysis

Glucose concentrations were determined using a colorimetric method with 3,5-dinitrosalicylic acid (DNS) (Miller 1959). A 1-mL portion of supernatant from a microcosm (or dilutions of the supernatant yielding 1 mL final volume) was mixed with 1 mL of DNS reagent and this was heated at 90°C for 5 min. After cooling, the absorbance at 540 nm was measured using a Philips Model PU 8740 UV/VIS scanning spectrophotometer.

The DNS reagent was prepared by dissolving 10 g DNS, 300 g potassium sodium tartrate and 16 g sodium hydroxide in 1 L of warm distilled water. The reagent was stored in a sealed dark glass container.

3.5.2 Nitrate analysis

A colorimetric assay was used to monitor the concentrations of nitrate in the anaerobic cultures that were incubated under nitrate-reducing conditions. The assay was modified from that reported by Cataldo et al. (1975) and is based on the principle that nitrate in the sample causes nitration of salicylic acid under acidic conditions. This causes the formation of a yellow compound that can be detected at a wavelength of 410 nm after the sample has been adjusted to pH >12.

The method was scaled-down so that only 0.1 mL of culture supernatant was needed for the analysis. To construct a calibration curve, this volume of standard nitrate solution, containing from 1 to 60 µg nitrate per 0.1 mL, was mixed with 0.4 mL salicylic acid (5% w/v) in concentrated H_2SO_4 and allowed to react at room temperature for 20 min. The reaction mixture was then placed on ice, and 9.5 mL of 2 M NaOH was added very slowly. After cooling, the absorbance of the yellow solution was measured at 410 nm. The method gave a calibration curve which was linear up to 300 mg nitrate/L.

3.5.3 Extractable Mn(II) analysis

As part of the measurement of microbial activity under Mn(IV)-reducing conditions, the microcosms were monitored for the production of Mn(II). Analyses were done using a Perkin-Elmer AAnalyst 700 Atomic Absorption Spectrophotometer (AA) equipped with a Mn lamp, measuring the absorbance at 279.5 nm. Manganese was added to microcosms as amorphous MnO_2 [Mn(IV)] (Lovley and Phillips, 1988). Although the form of manganese [Mn(II) or Mn(IV)] cannot be differentiated by AA, only Mn(II) is extracted into 0.5 M HCl (Lovley and Phillips, 1988). Thus, microcosm samples (0.1 mL of slurry) were added to 5 mL of 0.5 M HCl to extract the Mn(II). After 15 min, this was centrifuged and the supernatant was filtered (0.22 µm), diluted in $CaCl_2$ solution and then analyzed by AA to determine the amount of Mn(II) present.

Standard manganese solutions were prepared from a commercially-available manganese solution (Mn metal in 0.3 M HNO_3, 995 μg/mL). The typical calibration curve for manganese analysis was linear up to 8 mg Mn/L.

3.5.4 Soluble Fe(II) analysis

Numerous microorganisms have recently been identified which are capable of obtaining energy for growth by coupling the oxidation of organic compounds to ferric iron reduction. The most common way to measure this activity is to monitor the increase in ferrous iron [Fe(II)] over time using the ferrozine assay, as outlined by Lovley and Phillips (1986). Ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,3,5-triazine, reacts with divalent iron to form a stable magenta complex which is water-soluble and can be measured spectrophotometrically (Stookey, 1970). This assay is simple, quick, and reproducible, and has been used extensively by Gieg et al. (1998) and Greene et al. (1998) to monitor microbial Fe(III)-reducing activity.

Fe(II) standards for the ferrozine assay were prepared by dissolving 1.404 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 20 mL H_2SO_4 and 50 mL H_2O. The solution was brought to volume in a 100-mL volumetric flask, resulting in a 2000 mg/L stock Fe(II) solution. This stock solution was then diluted with distilled water into several standard solutions ranging from 0 to 50 mg Fe(II)/100 mL. Depending on the amount of Fe(II) to be assayed, 0.1 mL [containing 0 to 50 μg Fe(II)] or 1.0 mL [containing 0 to 500 μg Fe(II)] of these standard solutions could then be removed for reaction with ferrozine.

For the ferrozine assay, standards or culture samples (0.1 mL) were added to test tubes containing 5 mL of 0.5 M HCl. The tubes were vortexed and allowed to sit for at least 15 min to allow the dissolution of acid-soluble iron. Then, 0.1 mL of the acid solution was added to a separate test tube containing 5 mL of ferrozine (1 g/L) in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (at pH 7). The tubes were vortexed again and the absorbance at 562 nm was measured on a Philips Model PU 8740 UV/VIS scanning spectrophotometer.

Fe(II) standards were prepared as described above and 1.0 mL of each standard was analyzed by the ferrozine assay. The calibration curve obtained by plotting A_{562} vs amount of Fe(II) (0 to 500 μg) was linear.

3.5.5 Sulfate analysis

Sulfate analyses were done by ion chromatography was using a Dionex DX600 instrument with Ionpac AS9-HC (4 mm) and AG9-HC (4 mm) columns in series. The ASRS-Ultra (4 mm) suppressor was operated in the recycle mode The mobile phase was 9 mM carbonate with a flow rate of 1 mL/min. The sample loop size was 25 µL.

3.5.6 Sulfide analysis

The method of Ulrich et al. (1997) was used to measure the concentration of sulfide in the slurries in the microcosms. This is an adaptation of a similar method described by Fossing and Jørgensen (1989). Chromium reducible sulfides (including elemental sulfur and pyrite), and acid volatile sulfides (free sulfide and FeS) were released from the slurry as H_2S which was trapped in an anoxic zinc acetate solution that was prepared by adding 50 g of zinc acetate to 500 mL of freshly boiled water, and then sparging with O_2-free nitrogen while the solution cooled. One hundred millilitre portions of the solution were dispensed into 158-mL serum bottles that had been flushed with O_2-free nitrogen, and these were stoppered and sealed.

In preparation for the analysis, a small test tube was placed into a 120-mL serum bottle and the tube and bottle were flushed with O_2-free nitrogen While still flushing, 2.5 mL of anoxic zinc acetate solution were added to the test tube. Then the bottle was stoppered and sealed leaving the test tube inside. These were placed in an anaerobic chamber and weighed.

Each microcosms was shaken well, and a sample of slightly less that 2 g (approximately 1.5 mL) of slurry was removed with a syringe and this was quickly injected into the tared serum bottle, ensuring that none of the slurry was placed into the tube of zinc acetate. The wet weight of the slurry sample was recorded. Then, using a syringe, 8 mL of 1 M Cr(II)-HCl solution was added to the slurry sample. The Cr(II)-HCl solution was prepared by placing 200 g of mossy zinc in a large bottle. The zinc was covered with 0.5 M HCl and left to react until the zinc surface was clean and bright. Then the HCl was discarded, and the bottle was then flushed with O_2-free nitrogen. Separately, 133 g of $CrCl_3$ $6H_2O$ was dissolved in 500 mL of 0.5 M HCl. The Cr(III) solution was dark green. The solution was reduced by reacting it with the freshly prepared zinc while being continuously flushed with O_2-free nitrogen. After about 30 min, the solution became blue indicating the formation of Cr(II).

After adding the Cr(II)-HCl solution to the slurry sample, 4 mL of 12 M HCl were added with a syringe and then the bottle was placed on a shaker at 150 rpm for 48 h. During this time, the liberated sulfide formed H_2S which was trapped in the zinc acetate solution in the test tube. The test tube was removed from the serum bottle in order to determine the amount of sulfide that was trapped as insoluble zinc sulfide. This precipitate tended to stick to the glass wall of the test tube, making it difficult to obtain a representative sample. This problem was resolved by covering the test tube with a rubber cap, adding 4 drops of 0.5 M HCl to the tube with a syringe, and vortexing the tube. This washed the precipitate off the walls, and resulted in a pH around 5.6, which was well within the range for the test kit used for sulfide analysis. After vortexing, the rubber cap was removed and the sulfide concentration was measured using a CHEMetrics sulfide analysis kit (CHEMetrics, Calverton, VA). This analysis is based on the reaction of sulfide to produce methylene blue.

3.5.7 Methane analysis

Methane was analyzed with a Hewlett Packard model 5700A GC and the packed column (Budwill et al. 1996). Methane standards were prepared in rigid containers as outlined by Nelson (1971). Samples of 0.1 mL headspace gas from microcosms were analyzed by this method. Results were expressed as percent by volume, and these were not corrected for temperature, pressure or vapor pressure of water in the closed microcosms.

3.5.8 Soluble barium analysis

Barium analyses were done using a Perkin-Elmer AAnalyst 700 Atomic Absorption Spectrophotometer equipped with both a flame and a HGA graphite furnace system. Analyses were preformed using a barium hollow cathode lamp at 553.6 nm with a slit width of 0.2 low slit height. Absorption was measured as integration of peak area and barium concentrations were measured as μg/L.

The concentration of barium in solutions was measured using the protocol recommended by the manufacturer. Using the automated AA system, 20 μL of the sample in 0.2% (v/v) high purity nitric acid (Trace metal grade, Cat. no. A5095K-212 Fisher Scientific) was dried for 20 s at 100°C followed by 45 s at 170°C, pretreated for 25 s at 1200°C and then atomized for 5 s at 2550°C in a pyrolytically coated graphite tube.

Standards were prepared using a Ba reference solution (Certified reference standard solution, Fisher Scientific). All glassware was soaked in 5% high purity nitric acid and all plasticware was soaked in 0.2% high purity nitric acid prior to use. After 4 to 5 days of soaking, these were rinsed with deionized water (MilliQ grade) and dried before use. Supernatant samples from the microcosms were filtered through 13-mm diameter, (0.45-µm pore size) nitrocellulose filters (Millipore HAWP01300). Each filter was placed in reusable Swinnex (SX0001300) casing attached to a syringe that was used to force the sample through the filter to yield a particulate-free sample for AA analysis.

During the evaluation of the barium analysis method, some samples were ashed using a method adapted from McDonald-Stephens and Taylor (1995). Briefly, samples in borosilicate test tubes were dried at 55°C under a stream of air and then ashed at 500°C to drive off any potential interfering organic material. The residual ash was dissolved in 0.2% (v/v) high purity nitric acid and the barium concentration was determined by AA.

The amount of soluble Ba^{++} in the microcosms was expressed as "released Ba (mg/kg soil)" (wet weight). The slurry samples taken after 4 months of incubation were used to determine the wet weight of soil. The slurry was centrifuged at top speed in a clinical centrifuge for 5 min. All of the supernatant was removed, and the tube with the wet soil was weighed. Then the soil was quantitatively removed from the tube, and after the tube was washed and dried it was weighed. The mass of wet soil in the original slurry was the difference between the weights. Typically, the wet weights were between 1.7 and 2.5 g.

4 RESULTS AND DISCUSSION

4.1 Evaluation of analytical methods

Most of the analytical methods used in this study have been used extensively in previous investigations in our laboratory (Fedorak et al. 1997; Gieg et al. 1998; Greene et al. 1998), therefore they were used without further evaluation. Two exceptions were the analyses for sulfide and for soluble barium. These were both evaluated and the results are presented in the following two sections.

4.1.1 Sulfide analysis

The utility of the method used to release H_2S from chromium reducible sulfides and from acid volatile sulfides is well documented (Fossing and Jørgensen 1989; Ulrich et al. 1997). Thus we simply applied the method of Ulrich et al. (1997) to liberate H_2S from the slurry samples. We focused on the ability to recover liberated H_2S based on analyses with the CHEMetrics sulfide test kit which has been used successfully in our laboratory (Eckford and Fedorak 2002). The preparation of standard sulfide solutions is quite time consuming and these solutions must be prepared fresh each time samples are analyzed. By using the CHEMetrics sulfide test kit, there is no need to prepare standard sulfide solutions because the kit provides series of colored standards against which the reacted sample is compared. Although the method is very convenient, some accuracy is lost because of the limited range of colored standards provided. For example, two of the colored standards represent 30 mg/L and 40 mg/L sulfide. If the color of the reacted sample falls between these two standards, one must estimate the concentration within that range. However, strictly quantitative sulfide analyses were not needed for this project because we simply wanted to verify that sulfate reduction was occurring in the microcosms. An increase in sulfide concentration would verify sulfate reduction.

The method for sulfide analysis depended on recovering released sulfide as zinc sulfide in a 10% (w/v) zinc acetate solution. To evaluate the method, a fresh solution of 0.25 g of $Na_2S{\cdot}9H_2O$ in 10 mL of water was prepared. Then 0.1 mL of this solution was added to two test tubes each containing 9.9 mL of 10% (w/v) zinc acetate solution. The concentration of sulfide (precipitated as zinc sulfide) in each of the final solutions was calculated to be 33 mg/L. Using the CHEMetrics sulfide test kit, the concentration of sulfide was determined to be about 35 mg/L. This confirmed that the CHEMetrics method could accurately measure the sulfide concentration in a mixture of zinc acetate and zinc sulfide.

To evaluate the recovery of sulfide using the method of Ulrich et al. (1997), 2.5 mL of the zinc sulfide in zinc acetate solution (containing 33 mg sulfide/L) was added to each of two sealed serum bottles. Each serum bottle was anoxic, and contained a test tube with 2.5 mL of zinc acetate solution. After adding the Cr(II) solution and 12 M HCl, the serum bottles were placed on a shaker at 150 rpm for 48 h. If all of the sulfide in the serum bottle was recovered as zinc sulfide in the test tube, the concentration of sulfide in the test tubes would be near 33 mg/L. Results obtained with the CHEMetrics sulfide test kit, gave concentrations of about 34 and 35 mg/L in samples from the two serum bottles. These results indicated that the method used for sulfide determination was certainly adequate for this study.

4.1.2 Soluble barium analysis

Our laboratory had no previous experience doing soluble barium (Ba^{++}) analyses, thus various aspects were investigated to ensure accurate and reproducible results. The AA spectrophotometer automatically prepared dilutions of a standard Ba^{++} solution to generate calibration curves. The calibration was linear, with correlation coefficients typically >0.99.

New Swinnex filter casings (see section 3.4.8) were ordered and many samples and standards were filtered during preliminary studies (data not presented). Subsequent re-use of the filter casings suggested that residual barium remained on casings and that rigorous cleaning of the casings was required between uses. This was verified by filtering 5 mL of MilliQ water through three randomly chosen re-used filter casings and analyzing these for barium. Then 5 mL of 0.2% high purity nitric acid was filtered through the same filter casings and analyzed for barium. No barium was detected in two of the three samples of filtered MilliQ water. In the third sample of filtered MilliQ water, the concentration of barium was 1.5 μg/L. In contrast, barium was detected in all three portions of filtered nitric acid, with concentrations ranging from 8 to 15 μg/L. Thus, it was evident that simply rinsing the filter casings with MilliQ water before re-use would not adequately remove residual barium. Hence, the casings were cleaned by soaking in 0.2% high purity nitric acids for 4 to 5 days and then rinsing with copious amounts of MilliQ water.

To verify that Ba^{++} was not lost during filtration, a solution of approximately 50 μg Ba^{++}/L was prepared and analyzed in triplicate by AA. These analyses gave a mean concentration of 46±4.2 (standard deviation) μg/L. Then three portions of this solution were filtered using three nitric acid cleaned filter casings. AA analyses gave a mean of 44±1.4 μg/L. Thus, no significant amounts of Ba^{++} were lost during filtration. In addition, this experiment also demonstrated that the nitric acid cleaned filter casing did not contaminate the sample with residual barium from previous uses.

Slurry samples from the microcosms were centrifuged in a clinical centrifuge to provide supernatants for various analyses. It was postulated that this centrifugation might be adequate to remove the insoluble barium, leaving only Ba^{++} in the supernatant. Thus, sample filtration may not be required. To test this hypothesis, a slurry containing 50 g of background soil and 100 mg of barium sulfate in 100 mL of modified Butlin's medium was prepared in a serum bottle. Slurry samples were removed and centrifuged. Three portions of the supernatant were diluted 25-fold in MilliQ water and analyzed for barium. Three additional portions of the supernatant (5 mL each) were filtered using three nitric acid cleaned filter casings, and analyzed for barium after appropriate dilutions in MilliQ water. The mean concentration of barium in the samples that were centrifuged and filtered were 45±1.3 μg/L, whereas the mean concentration of barium in the samples that were only centrifuged were 72±1.6 μg/L. The higher concentration in the sample that was only centrifuged indicated that particulate barium sulfate contributed to the overall barium concentration. Therefore, centrifugation alone was not adequate to remove barium sulfate from the supernatant, and filtration was required to yield samples for Ba^{++} analysis.

The solubility product of barium sulfate is $1x10^{-10}$ (Weast 1970). Thus, at equilibrium, the concentration of Ba^{++} would be 10^{-5} M or 1.37 mg/L. The barium concentration in the 25-fold dilution of the centrifuged and filtered supernatant (described in the previous paragraph) was 45±1.3 μg/L. Thus, in the undiluted supernatant, the concentration was 1,100 μg/L or 1.1 mg/L. This value is in good agreement with the expected concentration calculated on the solubility product.

The presence of organic matter can interfere with the AA analysis of some metals (J.L. McDonald-Stephens, University of Alberta, personal communication), but the interference can be removed by ashing samples to remove the organic matter. Thus the need to ash samples for Ba^{++} analysis was evaluated. First, a 100 μg Ba^{++}/L solution was prepared and analyzed by AA (in triplicate). Three portions of this solution were put through the ashing procedure (McDonald-Stephens and Taylor 1995) and then analyzed by AA. The mean Ba^{++} concentration of the solution that was not ashed was 101±4.1 μg/L and concentration of the solution that was ashed was 102±1.2 μg/L. These results showed excellent recovery of Ba^{++} during the ashing procedure. Then, a slurry of 50 g of the contaminated soil was made in 100 mL of modified Butlin's medium. Slurry samples were removed and centrifuged yielding a supernatant that then was filtered. Three portions of supernatant were ashed analyzed by AA and two portions of supernatant were analyzed without being ashed. In each case, the portions were diluted 25-fold in MilliQ water prior to AA analysis. The mean concentration of Ba^{++} in the samples that were not ashed was 32±7 µg/L and the mean concentration of Ba^{++} in the samples that were ashed was 30±6.5 µg/L. These concentrations were essentially the same, indicating that the time-consuming ashing procedure was not required for the analyses of Ba^{++} in samples from the microcosms.

4.2 Results from microcosm studies

A variety of microcosms were established under several different redox conditions, based on the terminal electron acceptors supplied to the microcosms. In general, for a given electron donor (such as glucose), the energy available from microbial metabolism via redox processes with different electron acceptors are as follows: aerobic > nitrate-reducing > Mn(IV)-reducing > Fe(III)-reducing > sulfate-reducing > CO_2-reducing (methanogenic) conditions. The results from the microcosm studies are presenting in this order of decreasing available energy. In the figures presented in this report, the variability in the measured parameters is shown with error bars representing one standard deviation above and below the mean. In many cases, the plotted symbols are larger than the extent of the error bars.

4.2.1 Aerobic microcosms

It was hypothesized that no Ba^{++} would be released under aerobic conditions, so the aerobic microcosms were only incubated for 2 months, whereas the anaerobic microcosms were incubated for 4 months. Because aerobic metabolism of glucose, a readily utilizable growth substrate, was expected to be rapid, the microcosms were re-supplemented with glucose on five occasions over the 2-month incubation. Figure 4.1 shows the glucose concentrations in the aerobic microcosms, and these data demonstrate the rapid consumption of glucose by microorganisms in the background soil. No glucose was added to the sterile controls.

The aerobic cultures were incubated with vigorous shaking (at 200 rpm), and from previous experience, this provides ample O_2 to cultures. Therefore, the concentrations of dissolved oxygen (the electron acceptor under aerobic conditions) were not measured during this experiment.

At various times, slurry samples were removed from the microcosms, and the concentrations of soluble Ba^{++} in supernatants were measured. These values were divided by the wet weight of soil in the slurry sample taken at 2 months, and the results are shown as the "Released Ba (mg/kg soil)" in Fig. 4.2. The data show that the amounts of soluble Ba^{++} in the sterile control ranged from 4.4 to 6 mg/kg soil over the 2-month experiment. Surprisingly, the

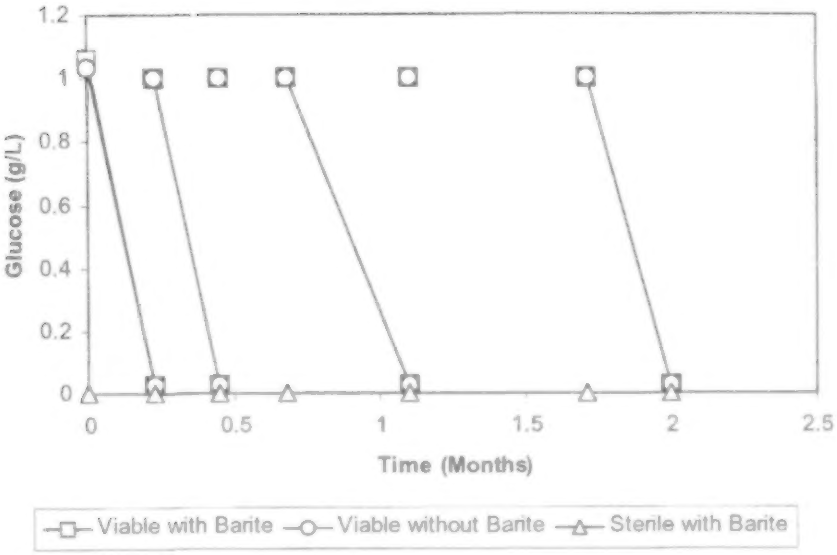

Fig. 4.1. Glucose concentrations in the aerobic microcosms. Concentrations of 1 g/L were estimated based on the amount of glucose solution added immediately after re-supplementing with at 0.2, 0.5, 0.7, 1.1, and 1.8 months. Error bars show one standard deviation.

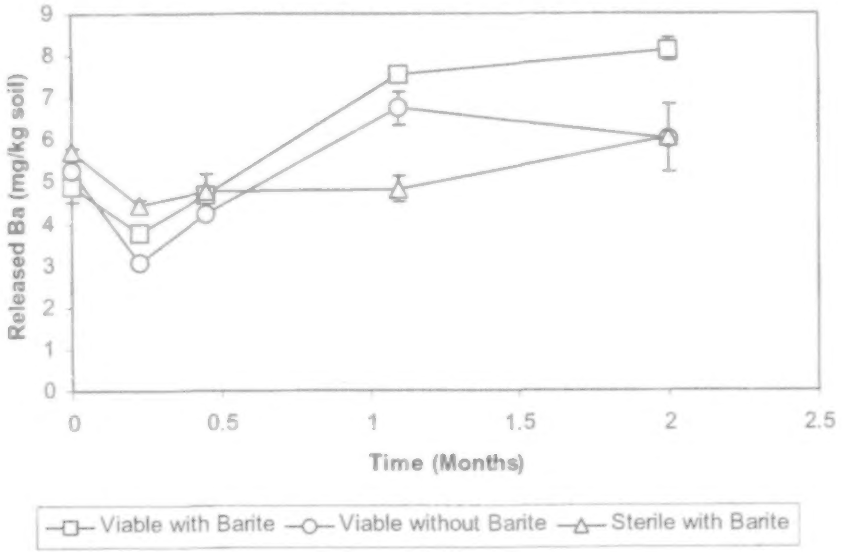

Fig. 4.2. Release of soluble Ba^{++} in the aerobic microcosms. Error bars show one standard deviation.

amounts of soluble Ba^{++} in the viable microcosms that were supplemented with 100 mg barite were higher than those in the sterile controls after 1 month and 2 months incubation (Fig. 4.2). The released Ba^{++} values were 7.5±0.2 and 8.1±0.3 mg/kg soil in the 1 month and 2 month samples, respectively. Based on t-tests, these values were statistically higher ($P<0.01$) than the corresponding control values.

The release of soluble Ba^{++} under aerobic conditions was unexpected because according to information in the literature, barite dissolution occurs under anaerobic conditions, mediated by sulfate-reducing bacteria (McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). However, in a nearly unintelligible report, Baldi et al. (1996) described an experiment in which barite-contaminated anaerobic sewage sludge was aerated and barium concentrations in the suspended matter were monitored. They wrote, "The experiment demonstrated that there was an 'oxidizable' fraction of barium compounds in the sewage sludge which explains the finding of lower dissolved Ba concentration in the oxidized suspended matter." This statement seems to indicate that soluble Ba^{++} was released during this 100-h experiment. We are not aware any other report of barite dissolution under aerobic conditons.

The mechanism leading to soluble Ba^{++} release in our aerobic microcosms in unknown. However, the process appears to be microbially-mediated because there was very little change in the amount of soluble Ba^{++} in the sterile controls (Fig. 4.2). The release of soluble Ba^{++} was most evident in the viable microcosms that were supplemented with barite and glucose.

4.2.2 Nitrate-reducing microcosms

Figure 4.3 summarizes the nitrate concentrations in these microcosms that contained background soil. The initial nitrate concentrations in the viable microcosms with barite and in the viable microcosms without barite were 3.1±0.0 g/L and 3.5±0.1 g/L, respectively. After 2 weeks incubation, the corresponding nitrate concentrations were 2.0±0 g/L and 2.1±0 g/L. The sterile controls (that contained 100 mg barite) were not supplemented with glucose to minimize the chance of microbial growth if they were contaminated during sampling. There was no evidence of nitrate reduction in the controls because the nitrate concentration remained at about 3.4 g/L over the 4 months of incubation the (Fig. 4.3). The nitrate concentrations in the two sets of viable cultures decreased at essentially the same rate and by month 3, the nitrate was depleted.

The initial glucose concentrations in the viable microcosms with 100 mg barite and in the viable microcosms without barite were 0.93 g/L and 0.94 g/L, respectively (Fig. 4.4). After 2 weeks of incubation, no glucose was detected in any of the viable microcosms and there was virtually no nitrate consumption between 2 weeks and 1 month of incubation (Fig. 4.3)

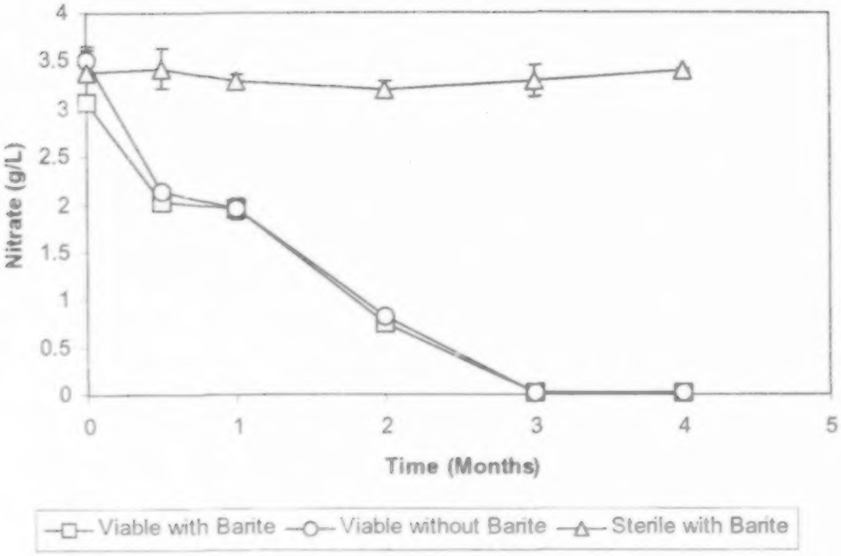

Fig. 4.3. Nitrate concentrations in the nitrate-reducing microcosms. Error bars show one standard deviation.

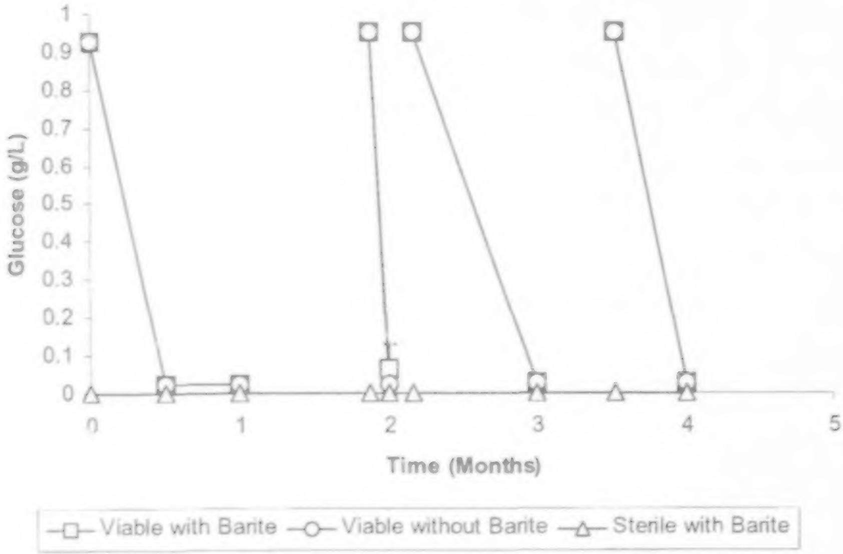

Fig. 4.4. Glucose concentrations in the nitrate-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.9, 2.2, and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

because glucose had been depleted. To maintain the nitrate-reducing activity, the viable microcosms were re-supplemented with glucose after 57, 67, and 109 days (1.9, 2.2 and 3.6 months, respectively) of incubation (Fig. 4.4).

The results in Figs. 4.3 and 4.4 clearly indicated that the electron donor (glucose) was being consumed at the expense of the electron acceptor (nitrate). In addition, there was an increase in gas pressure in the viable microcosms, which is consistent with nitrate reduction leading to N_2 production during denitrification.

The release of soluble barium is summarized in Fig. 4.5. Surprisingly, there was an increase in the amount of soluble Ba^{++} in the "sterile" controls after 1 month of incubation. Despite the repeated autoclaving steps and the addition of chloroform to these microcosms, it appears that some microbial activity occurred in these microcosms. Figure 4.3 shows there was no consumption of nitrate in the "sterile" controls. Thus, the microbial contaminants in the "sterile" controls were likely not nitrate-reducing bacteria. The release of Ba^{++} suggests that sulfate-reducing bacteria were active in the "sterile" controls. It is often very difficult to kill all microorganisms in soil, and some genera of sulfate-reducing bacteria form endospores. These heat-resistant forms may have survived autoclaving. None of the other sterile controls prepared and monitored in this study appeared to have been contaminated with microorganisms that would release soluble Ba^{++} from barite.

Nonetheless, there was no evidence of the release of soluble barium in the viable cultures (Fig. 4.5) that consumed nitrate (Fig. 4.3) and glucose (Fig. 4.4). There is a considerable amount of information in the literature (Poduska and Anderson 1981; Jenneman et al. 1986; McInerney et al. 1996; Londry and Suflita 1999; Eckford and Fedorak 2002) showing that in the presence of nitrate, nitrate-reducing bacteria out-compete sulfate-reducing bacteria and retard sulfate reduction. Laboratory (Londry and Suflita 1999; Nemati et al 2001; Eckford and Fedorak 2002) and field (Jenneman et al. 1997) studies have demonstrated that nitrate-reducing bacteria can be stimulated in oilfield waters by the addition of nitrate. This process is used to control oilfield souring caused by microorganisms. This same mechanism likely occurred in the glucose- and nitrate-supplemented microcosms, preventing release of soluble barium in the viable cultures (Fig. 4.5) because of the stimulated activities of nitrate-reducing bacteria.

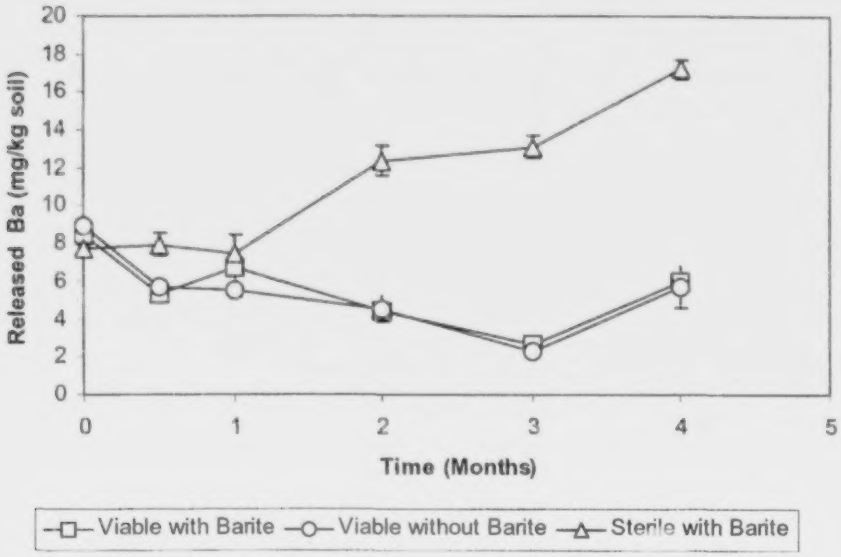

Fig. 4.5. Release of soluble Ba^{++} in the nitrate-reducing microcosms. The "sterile" controls appear to have been contaminated with sulfate-reducing bacteria. Error bars show one standard deviation.

4.2.3 Mn(IV)-reducing microcosms

Like the nitrate-reducing microcosms, the Mn(IV)-reducing microcosms contained background soil. There appeared to be no Mn(IV)-reducing activity in the sterile control, and the soluble Mn(II) concentration remained essentially constant near 0.6 g/L (Fig. 4.6). In contrast, there was an increase in the concentration of soluble Mn(II) from 0.5 to 1.9 g/L in the viable microcosms over the 4-month incubation period (Fig. 4.6). The amount of Mn(II) formed was independent of the presence of added barite in the microcosms.

Glucose was consumed rapidly in the Mn(IV)-reducing microcosms (Fig. 4.7). The initial glucose concentration in the microcosms with and without added barite were 0.95 g/L and 0.99 g/L, respectively. All the glucose was depleted after 2 weeks incubation. These microcosms were re-supplemented with glucose after 42, 52, and 93 days (1.4, 1.7, and 3.1 months, respectively) of incubation. The added glucose was consumed by the next time the microcosms were analyzed for glucose concentrations. No glucose was added to the sterile controls. The increases in the consumption of glucose and the increase in soluble Mn(II) concentrations indicate that Mn(IV) reduction occurred in the viable microcosms.

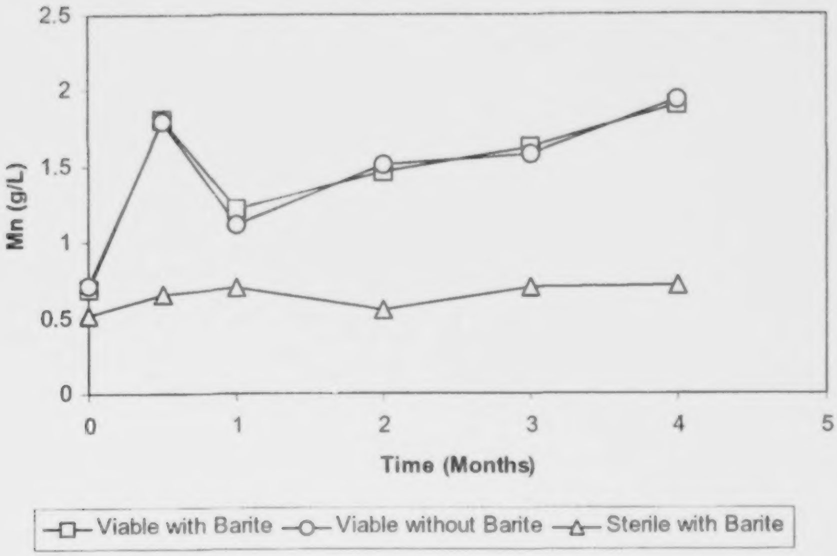

Fig. 4.6. Soluble Mn(II) concentrations in the Mn(IV)-reducing microcosm. Error bars are one standard deviation.

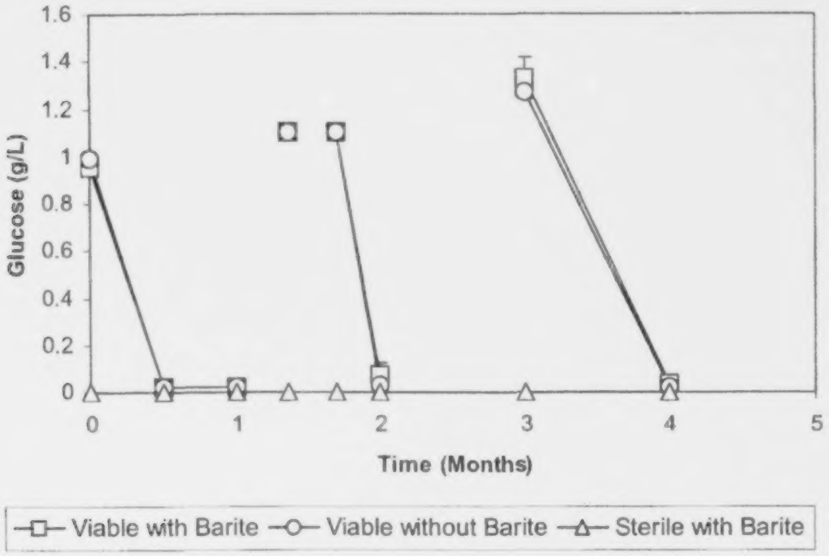

Fig. 4.7. Glucose concentrations in the Mn(IV)-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.4 and 1.7 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Figure 4.8 shows that under Mn(IV)-reducing conditions, soluble Ba^{++} was released in the microcosms that were supplemented with barite. The rate of soluble Ba^{++} release in these microcosms was nearly linear (Fig. 4.8), and occurred concurrently with Mn(IV) reduction (Fig. 4.6).

The amount of soluble Ba^{++} increased in each of the three replicate microcosms, but the amount of release in one of these was considerably lower than in the other two replicate microcosms. This variability led to the large error bars in the 3 and 4 month averages (Fig. 4.8). However, at 4 months, the amount of soluble Ba^{++} in the viable microcosm that gave the least amount of soluble Ba^{++} release was 28 times greater than the average amount in the sterile controls.

The average amounts of soluble Ba^{++} in the barite-supplemented, viable microcosms were compared to those in the sterile controls. There was significantly more soluble Ba^{++} in the viable microcosms after 3 months of incubation ($P<0.05$), however, because of the large variability in the data, there was no statistical difference after 4 months of incubation. After 3 months of incubation, there was a 36-fold increase in the average amount of released soluble Ba^{++} in the barite-supplemented microcosms (Fig. 4.8).

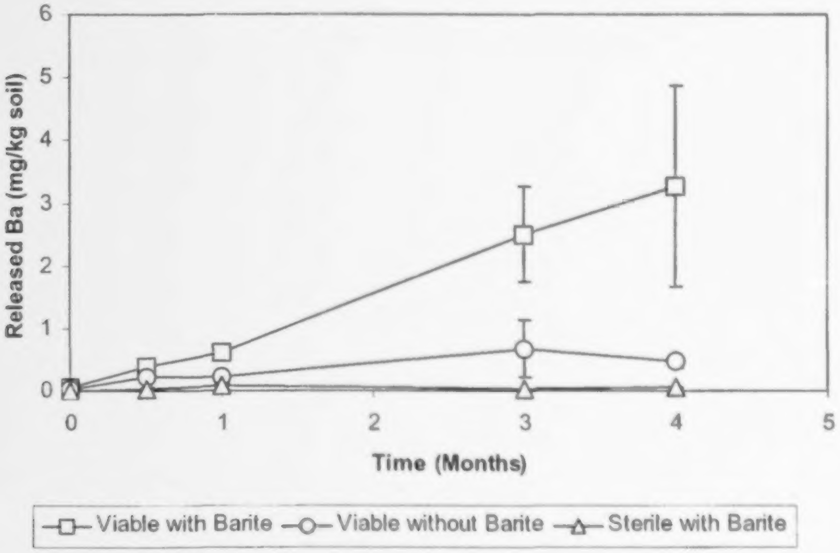

Fig. 4.8. Release of soluble Ba^{++} in the Mn(IV)-reducing microcosm. Error bars are one standard deviation.

The simultaneous release of soluble Mn(II) and Ba^{++} in these microcosms is consistent with the field observations of Martin et al. (2003) who studied the release of ^{226}Ra^{++} from (Ba,Ra)SO_4

sediments in a disposal pond at a Canadian uranium mine. They reported elevated concentrations of soluble Mn(II) and Ba^{++} at about the same depths of the sediments in the pond. Thus, it appears that Ba^{++} release can occur while active Mn(IV) reduction occurs.

4.2.4 Fe(III)-reducing microcosms

The product of Fe(III) reduction is Fe(II). The concentrations of Fe(II) increased with time in the viable microcosms that contained background soil (Fig. 4.9). The initial concentration was 0.2 g/L and after 4 months of incubation, this increased to near 4 g/L in the viable microcosms. The Fe(II) concentration in the sterile control remained constant at <0.1 g/L over this incubation time.

Glucose consumption was also observed in the Fe(III)-reducing microcosms (Fig. 4.10). Initially the microcosms with and without added barite contained glucose concentrations of 1.1 g/L. No glucose was detected after 2 weeks of incubation. The microcosms were re-supplemented with glucose after 41, 51, and 92 days (1.4, 1.7, and 3.1 months, respectively) of incubation. The consumption of glucose and the formation of Fe(II) indicated that microbial Fe(III) reduction occurred in the viable microcosms.

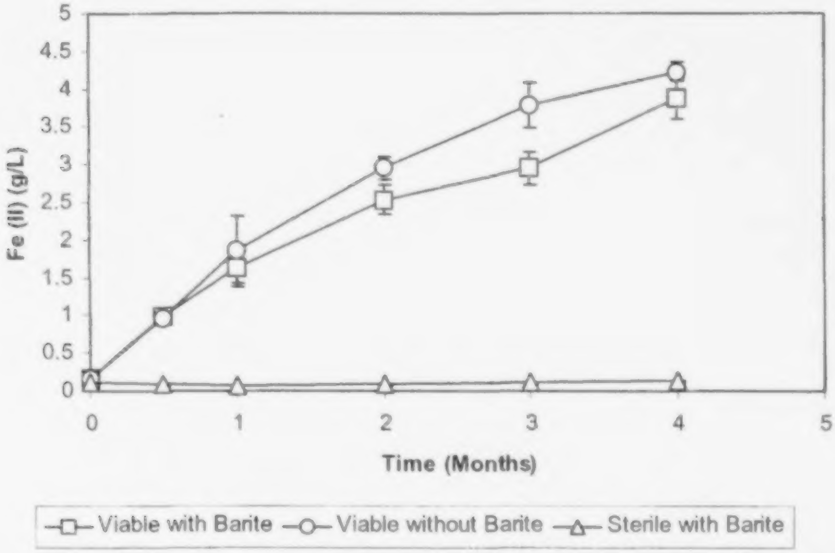

Fig. 4.9. Soluble Fe(II) concentrations in the Fe(III)-reducing microcosms. Error bars show one standard deviation.

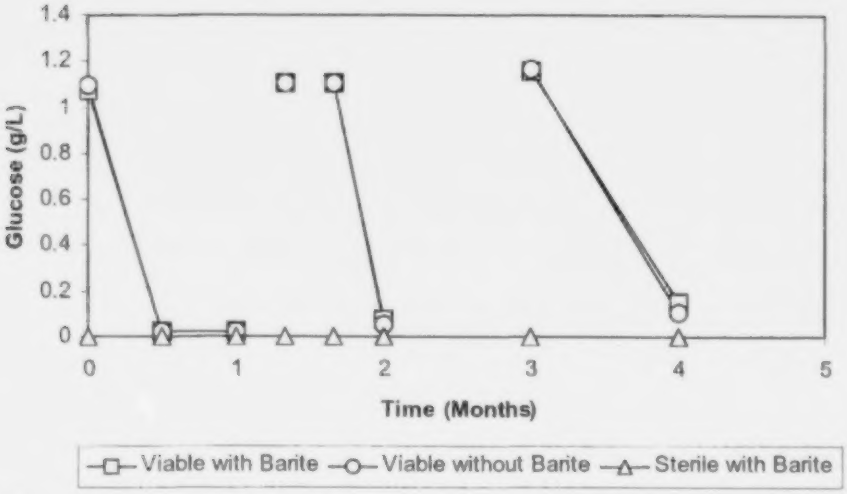

Fig. 4.10. Glucose concentrations in the Fe(III)-reducing microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.4 and 1.7 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

The release of soluble Ba^{++} was observed in microcosms that were incubated with and without added barite (Fig. 4.11). Over the 4-month incubation period, the amount of soluble Ba^{++} remained essentially constant in the sterile control, but increased 10-fold in the viable microcosms without added barite, and 43-fold in the viable microcosms with added barite. Based on t-tests, the amounts of soluble Ba^{++} in the viable microcosms were significantly greater ($P<0.01$) than in the sterile controls after 4 months incubation.

The results in Fig. 4.11 suggested that sulfate reduction might have occurred in the Fe(III)-reducing microcosms. Thus, sulfide analyses were done on the samples that were removed from these microcosm after 3 months of incubation. The sterile controls contained 3.1±0.9 μg sulfide/g soil, whereas the viable culture that was supplemented with barite contained 12±3 μg sulfide/g soil. These results indicate that sulfate reduction occurred in the Fe(III)-reducing microcosms. Fig. 4.11 shows that concentration of soluble Fe(II) increased between 1 and 3 months of incubation, thus the supply of added Fe(III) could not have been depleted during this time. This implies that both sulfate reduction and Fe(III) reduction occurred simultaneously in these microcosms.

In their studies of the release of $^{226}Ra^{++}$ from $(Ba,Ra)SO_4$ sediments in a disposal pond at a Canadian uranium mine, Martin et al. (2003) observed elevated concentrations of dissolved iron

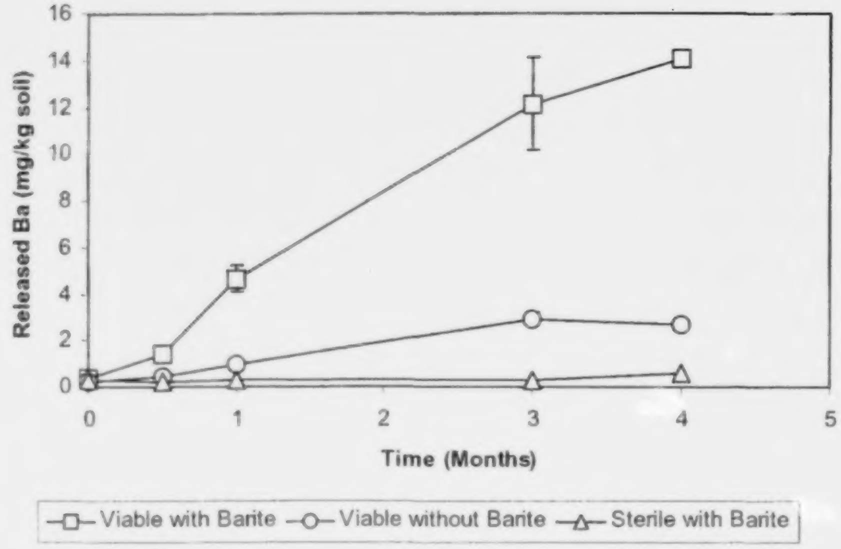

Fig. 4.11. Release of soluble Ba^{++} in the Fe(III)-reducing microcosms. Error bars show one standard deviation.

at depths where there were increased concentrations of dissolved $^{226}Ra^{++}$ and Ba^{++}. Similarly, Ulrich et al. (2003) reported an increased soluble Fe(II) and Ba^{++} concentrations downgradient from the Norman Landfill in Oklahoma. These field results suggest that Fe(III) reduction and sulfate reduction can occur in the same vicinities. Our microcosm studies show that they can occur in the same soil sample if it contains Fe(III) and sulfate to serve as terminal electron acceptors. We speculate that in the microcosms, Fe(III) reduction and sulfate reduction may be the result of different microbial populations co-existing in the soil. However, Coleman et al. (1993) demonstrated that under conditions in which the electron donor H_2 was abundant, the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, simultaneously reduced Fe(III) and sulfate.

4.2.5 Sulfate-reducing microcosms

Several reports in the literature have demonstrated that soluble Ba^{++} is released from barite under sulfate-reducing conditions (McCready et al. 1980; Fedorak et al. 1984; Phillips et al. 2001; Ulrich et al. 2003). Thus, a variety of microcosms were established under sulfate-reducing conditions to serve as positive controls. That is, to ensure that soluble Ba^{++} release could be detected using our experimental protocols. Although the results in some of the previous sections have demonstrated that barite dissolution occurs under sulfate-reducing conditions, the results from the sulfate-reducing microcosms are presented below for completeness.

Preliminary work with the two soil samples showed that the number of sulfate-reducing bacteria in the background soil was very low (9/g wet weight). Thus, sulfate-reducing bacteria were enriched from the contaminated soil, and these were added to 24 microcosms that contained background soil. In addition, microcosms that were not amended with the enrichment culture of sulfate-reducing bacteria were also established. These contained either background soil or contaminated soil. The results from the two sets of microcosms (with or without added sulfate-reducing bacteria) are presented separately in the following two sub-sections.

4.2.5.1 Microcosms containing native sulfate-reducing bacteria

Sulfide production was observed in microcosms that contained background soil (Fig. 4.12) or contaminated soil (Fig. 4.13). Over the 4-month incubation, there was little change in the sulfide found in the sterile controls, and the addition of glucose stimulated sulfide production in both soils. The microbial community in the contaminated soil released much more sulfide than did the community in the uncontaminated soil. The concentration of sulfide in the contaminated soil slurry increased from 19 to 71 μg/g in the glucose supplemented microcosms (Fig. 4.13). In contrast, the concentration of sulfide in the background soil slurry increased from 2 to 9.6 μg/g in the glucose supplemented microcosms (Fig. 4.12). These results demonstrated that both soils had active, native sulfate-reducing bacteria.

Glucose was consumed quickly in the microcosms that contained either background soil (Fig. 4.14) or contaminated soil (Fig. 4.15). From initial concentrations of near 1 g/L, no glucose was detected after 2 weeks of incubation. All of these microcosms were re-supplemented with glucose after 25, 35, and 77 days (0.7, 1.2, and 2.6 months, respectively) of incubation.

Some of these microcosms were supplemented with lactate, rather than glucose. However, lactate analyses were not done on the supernatants from the microcosms. Nonetheless, these microcosms were re-supplemented with lactate after 35 and 77 days of incubation.

The microcosms with the viable native sulfate-reducing bacteria released soluble Ba^{++} from barite. The amount of Ba^{++} released from the glucose- and barite-supplemented microcosms containing uncontaminated soil peaked at 13 mg/kg soil (wet weight) after 2 months (Fig. 4.16) this decreased to 7.8 mg/kg soil at the end of the 4-month incubation period. Based on t-tests, both of these values were greater than those found in the controls incubated for the same time ($P<0.01$).

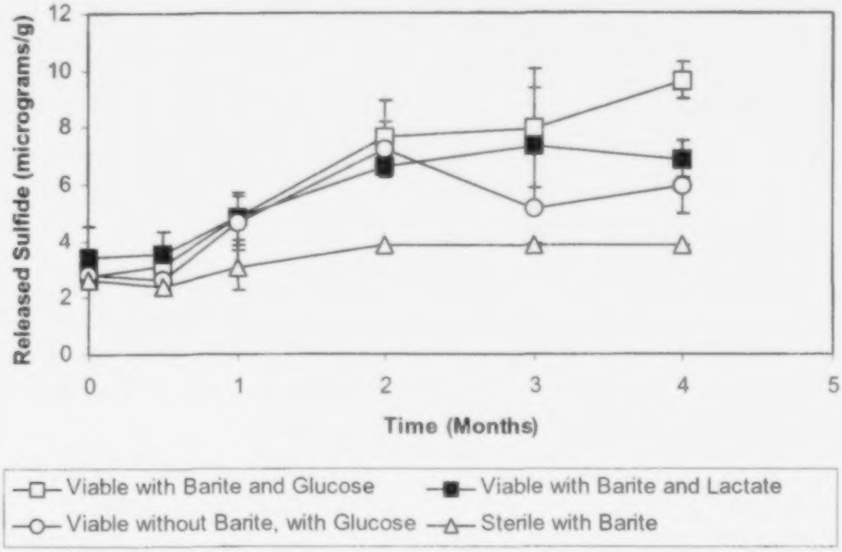

Fig. 4.12. Sulfide production in sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Error bars are one standard deviation.

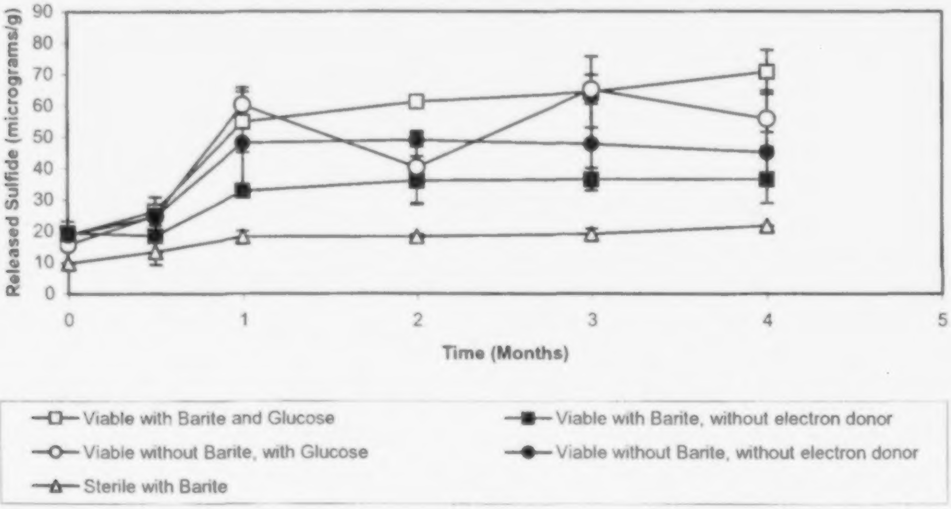

Fig. 4.13. Sulfide production in sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Error bars are one standard deviation.

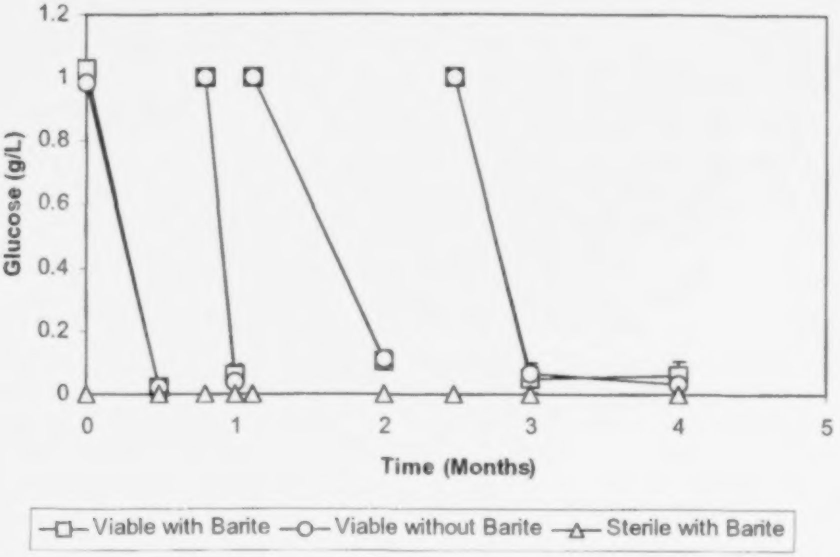

Fig. 4.14. Glucose concentrations in the sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Glucose concentrations immediately after re-supplementing with glucose at 0.7, 1.2, and 2.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

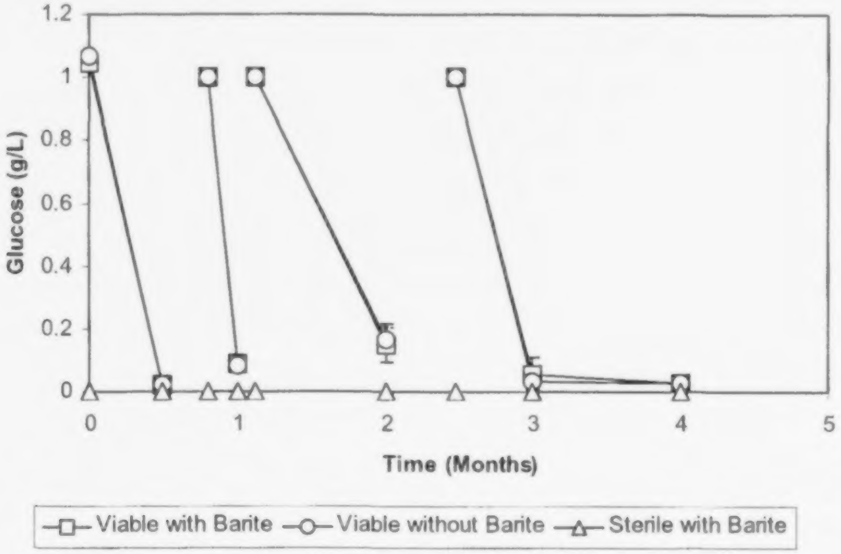

Fig. 4.15. Glucose concentrations in the sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Glucose concentrations immediately after re-supplementing with glucose at 0.7, 1.2, and 2.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Lactate is commonly used by many sulfate-reducing bacteria. Surprisingly, the addition of lactate to the background soil did not stimulate Ba^{++} release as quickly as did the addition of glucose (Fig. 4.16). However, the production of sulfide was observed in these microcosms (Fig. 4.12).

When supplemented with glucose, the microbial community in the contaminated soil released soluble Ba^{++} from barite (Fig. 4.17). The contaminated soil contained barite from drilling mud, and by the end of the 4-month incubation, the amount of soluble Ba^{++} released in viable microcosms with or without added barite were essentially the same (13 mg/kg soil). This value was significantly ($P<0.05$) higher than those in the viable microcosms without glucose (3.7 mg/kg soil) and in the sterile controls (0.7 mg/kg soil).

The barite-contaminated soil also had a much higher count of sulfate-reducing bacteria (9.3×10^3/g wet weight) than the background soil (9/g wet weight). The initial sulfide content was also higher in the contaminated soil-containing microcosms than in the background soil-containing microcosms. These concentrations were 19 µg/g (Fig. 4.13) and 3 µg/g (Fig. 4.12), respectively. The higher number of sulfate-reducing bacteria and the higher initial sulfide concentrations are consistent with active sulfate reduction occurring *in situ* in the contaminated soil. However, the initial soluble Ba^{++} concentrations in the time zero samples from the microcosms that contained contaminated soil were essentially the same as those from the microcosms that contained background soil. These concentrations were near 3 mg/kg soil (Figs. 4.16 and 4.17).

4.2.5.2 Microcosms supplemented with an enrichment culture of sulfate-reducing bacteria

Because of the low numbers of sulfate reducers in the background soil, 24 microcosms were supplemented with 9 mL of a cell suspension that contained 2.3×10^6 sulfate-reducing bacteria/mL. After supplementation, the number sulfate reducers was equivalent to 4.1×10^5/g (wet weight) of soil. The enrichment culture was grown with lactate as the major carbon and energy source, so lactate, rather than glucose, was added to the microcosms as the electron donor. These microcosms were re-supplemented with lactate after 28 and 70 days of incubation.

Two sets of microcosms were established to determine if the concentration of barite influenced the microbial activities. One set of microcosms was supplemented with 2000 ppm barite (the amount used in all of the previously discussed microcosms), and the second set was

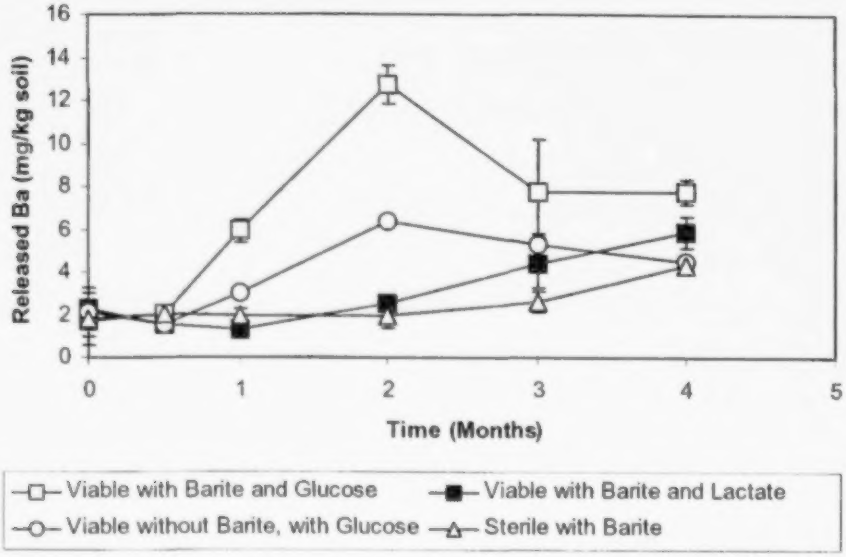

Fig. 4.16. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil and native sulfate-reducing bacteria. Error bars show one standard deviation.

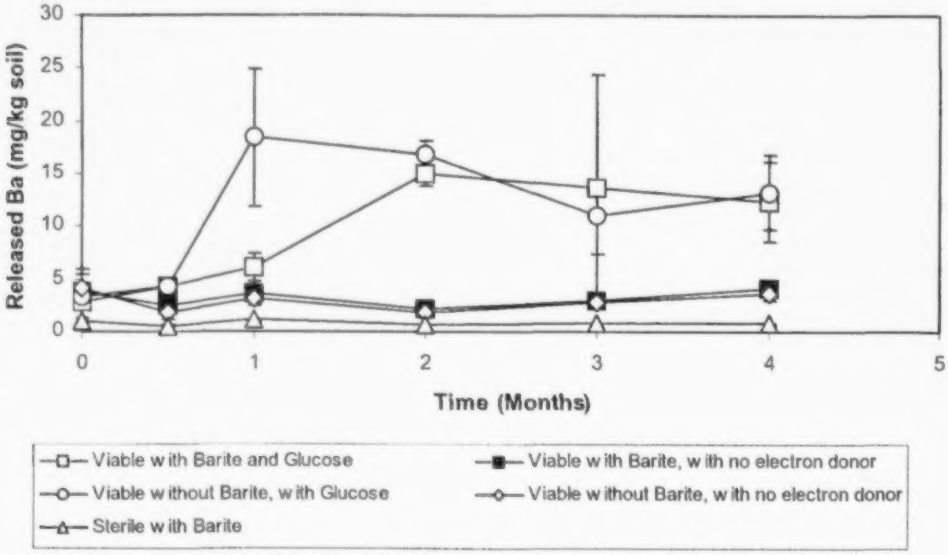

Fig. 4.17. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing contaminated soil and native sulfate-reducing bacteria. Error bars show one standard deviation.

supplemented with 10,000 ppm barite. Sulfide production and release of soluble Ba^{++} were observed in both sets of microcosms. Figs. 4.18 and 4.19 summarizes the sulfide production in these microcosms.

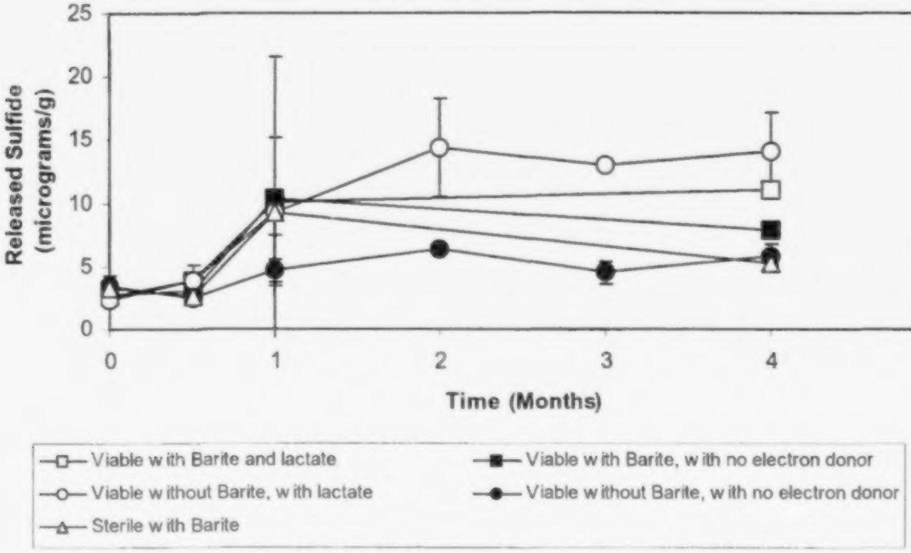

Fig. 4.18. Sulfide production in sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 2000 ppm barite. Error bars are one standard deviation.

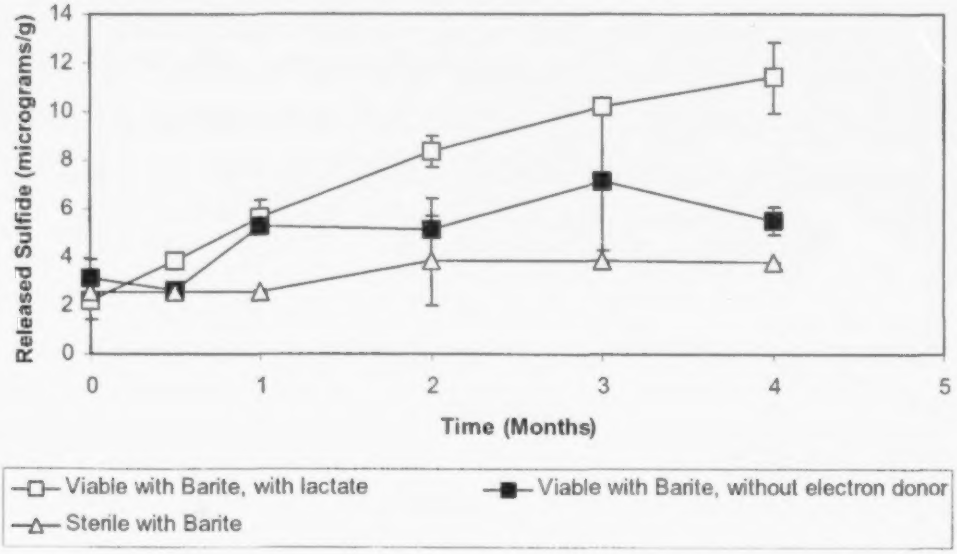

Fig. 4.19. Sulfide production in sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 10,000 ppm barite. Error bars are one standard deviation.

Considering the data after 4 months incubation, the sulfide concentration in the viable culture supplemented with lactate and 2000 ppm barite was significantly greater than that in the sterile control ($P<0.01$). Fig. 4.18 shows these concentrations to be 11±0.7 and 5.2±0.1 μg/g soil, respectively. Similarly after 4 months incubation, the sulfide concentration in the viable culture supplemented with lactate and 10,000 ppm barite was significantly greater than that in the sterile control ($P<0.01$). Figure 4.19 shows these concentrations were 11±1.5 and 3.2±0.01 μg/g soil, respectively. The different amounts of barite added to the microcosms did not affect the final sulfide concentrations in these microcosms. After 4 months incubation, those that contained 2000 ppm barite had 11±0.7 μg sulfide/g soil while those that contained 10,000 ppm had 11±1.5 μg sulfide/g soil. The microbial activity was likely limited by the amount of electron donor (lactate) rather than the amount of electron acceptor (sulfate from barite).

Considering the soluble Ba^{++} release data obtained after 4 months incubation, the release in the viable culture supplemented with lactate and 2000 ppm barite was significantly greater than that in the sterile control ($P<0.05$) (Fig. 4.20). These values were 6.0±0.8 and 3.4±0.3 mg/kg soil, respectively. Similarly after 4 months incubation, the soluble Ba^{++} release in the viable culture supplemented with lactate and 10,000 ppm barite was significantly greater than that in the sterile control ($P<0.01$) (Fig. 4.21). These concentrations were 6.9±0.6 and 2.4±0.3 mg/kg soil, respectively (Fig. 4.21). Thus, the different amounts of barite added to the microcosms did not affect the final soluble Ba^{++} release in these microcosms. After 4 months incubation, those that contained 2000 ppm barite released 6.0±0.8 mg Ba^{++}/g soil while those that contained 10,000 ppm released 6.9±0.6 mg Ba^{++}/g soil. As was noted with the discussion of sulfide production, the microbial activity in these microcosms was likely limited by the amount of electron donor (lactate) rather than the amount of electron acceptor (sulfate from barite).

4.2.6 Methanogenic microcosms

At the time this study was being planned, it was speculated that the background soil would contain few, if any, methanogens. Thus, two sets of methanogenic microcosms were established. One set with background soil that was supplemented with 10 mL of domestic anaerobic sewage sludge, and one set with only the background soil. Both sets were supplemented with 2000 ppm barite.

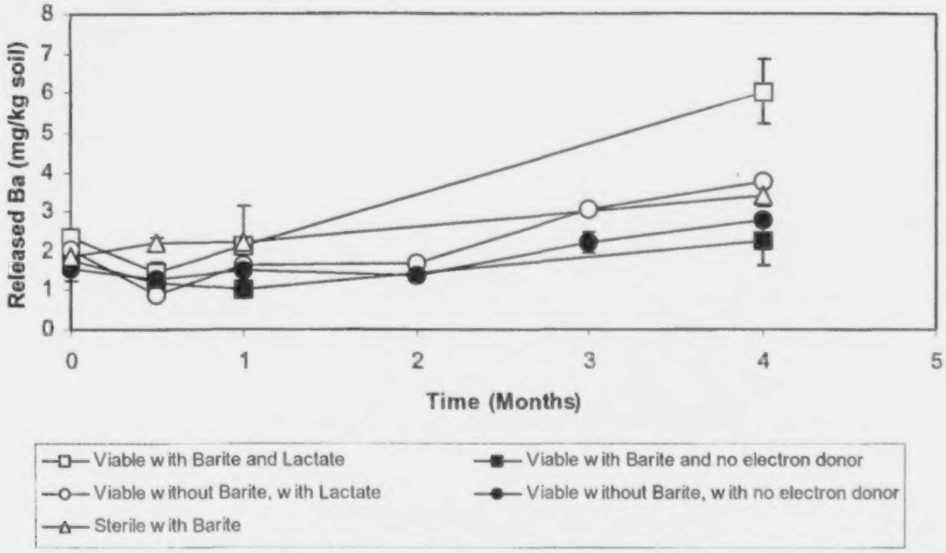

Fig. 4.20. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 2000 ppm barite. Error bars show one standard deviation.

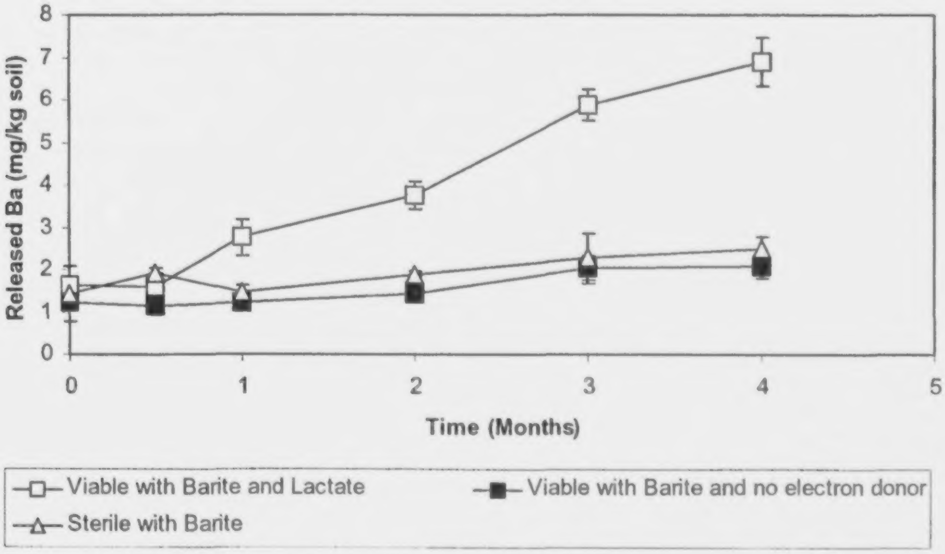

Fig. 4.21. Release of soluble Ba^{++} in the sulfate-reducing microcosms containing background soil supplemented with sulfate-reducing bacteria and 10,000 ppm barite. Error bars show one standard deviation.

4.2.6.1 Microcosms supplemented with anaerobic sewage sludge

As was observed with the other microcosms, glucose was consumed quickly in the methanogenic microcosms (Fig. 4.22). No glucose was detected in sewage sludge-supplemented methanogenic microcosms after 14 days of incubation. The viable microcosms were re-supplemented with glucose after 55, 65, and 107 days (1.8, 2.2, and 3.6 months, respectively) of incubation.

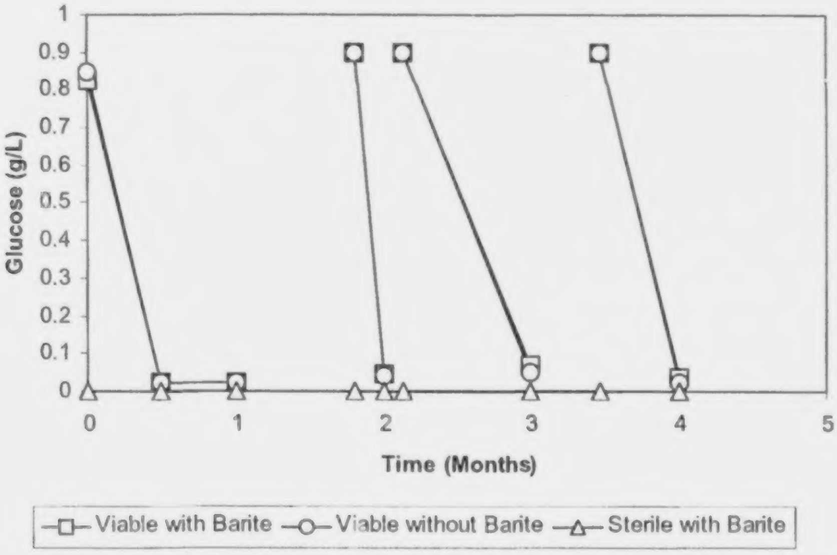

Fig. 4.22. Glucose concentrations in sewage sludge-supplemented microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.8, 2.2, and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

Methane production was measured to follow the activity of the methanogens. The results are shown in Fig. 4.23 in which the amount of methane is given as its volume percent in the headspace gas. Methane was detected on day 1 in the microcosms that were supplemented with sewage sludge. The results in Fig. 4.23 clearly show that methane production was reduced in the microcosms that contained barite. It is well documented that sulfate-reducing bacteria out-compete methanogens for substrates (Banat et al. 1983; Winfrey and Ward 1983). Sulfate reduction is thermodynamically (Karhadkar et al. 1987) and kinetically (Kristjansson et al. 1982; Schönheit et al. 1982) more favorable than methanogenesis. The addition of the barite to the control soil provided an electron acceptor for the sulfate-reducing bacteria, and they have diverted the flow of electrons away from methane production. Thus, the methanogenic microcosms that were supplemented with barite were likely behaving as sulfate-reducing microcosms.

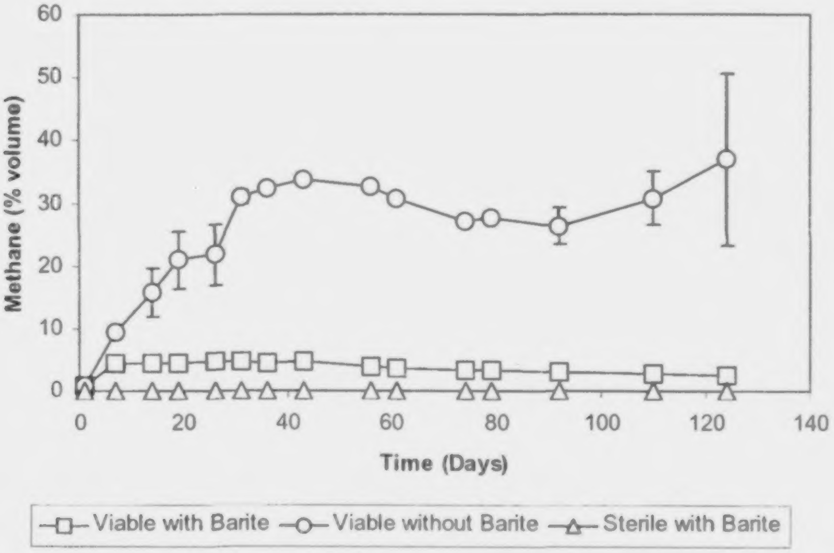

Fig. 4.23. Methane concentrations in sewage sludge-supplemented microcosms containing background soil. Error bars show one standard deviation.

The original plans for monitoring the methanogenic microcosms did not include measuring sulfide production. However, because of the observed inhibition of methane production caused by the addition of barite (Fig. 4.23), samples collected after 3 and 4 months of incubation were analyzed for sulfide. After 3 months of incubation, the average sulfide concentrations in the sterile, sewage sludge-supplemented methanogenic microcosms was 2.6±0.05 μg/g (wet weight), and in the viable sewage sludge-supplemented methanogenic microcosms, containing barite, was 6.0±0.7 μg/g (wet weight). There was a significant difference ($P<0.01$) between these means based on the t-test. The sulfide analyses clearly indicate that sulfate reduction occurred in the "methanogenic microcosms", and supports the notion that the sulfate-reducing bacteria were out-competing the methanogens for electron donors. However, after 4 months of incubation, the average sulfide concentrations in the sterile methanogenic microcosms was 3.8±0.06 μg/g (wet weight), and in the viable sewage sludge-supplemented methanogenic microcosms, containing barite, was 5.9±1.5 μg/g (wet weight). Because of the high variability in the results from the viable microcosms, the t-test indicated that the means were not significantly different.

Consistent with the reduction of sulfate in these microcosms, soluble Ba^{++} was released in the sewage sludge-supplemented microcosms that contained barite (Fig. 4.24). This was most obvious after 4 months of incubation when the average release was 5.4±0.3 mg/kg soil (wet weight) in the viable microcosms, and only 2.4±0 mg/kg soil (wet weight) in the sterile controls. Based on the t-test, there was a highly significant difference between these means ($P<0.001$). Thus, the results from the microcosms that were supplemented with anaerobic sewage sludge indicate that soluble Ba^{++} can be released from barite.

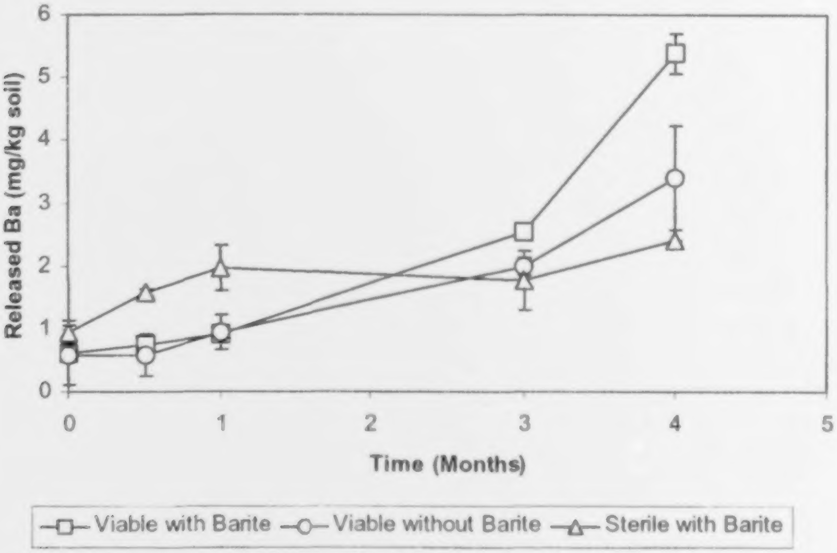

Fig. 4.24. Release of soluble Ba^{++} in the sewage sludge-supplemented microcosms containing background soil. Error bars show one standard deviation.

4.2.6.2 Microcosms with native methanogens only

Glucose was rapidly consumed in these methanogenic microcosms (Fig. 4.25) and by day 14, none was detected in the supernatant. The viable microcosms were re-supplemented with glucose after 55, 65, and 107 days (1.8, 2.2, and 3.6 months) of incubation.

The data in Fig. 4.26 showed that the background soil did contain methanogens, because methane production was evident after 40 days of incubation. The number of methanogens was likely quite low in this soil because of the long lag time before methane was observed compared to the microcosms that were supplemented with anaerobic sewage sludge in which methanogens were abundant. The lag period before methane was observed in the sewage sludge-supplemented microcosms was less than 1 day.

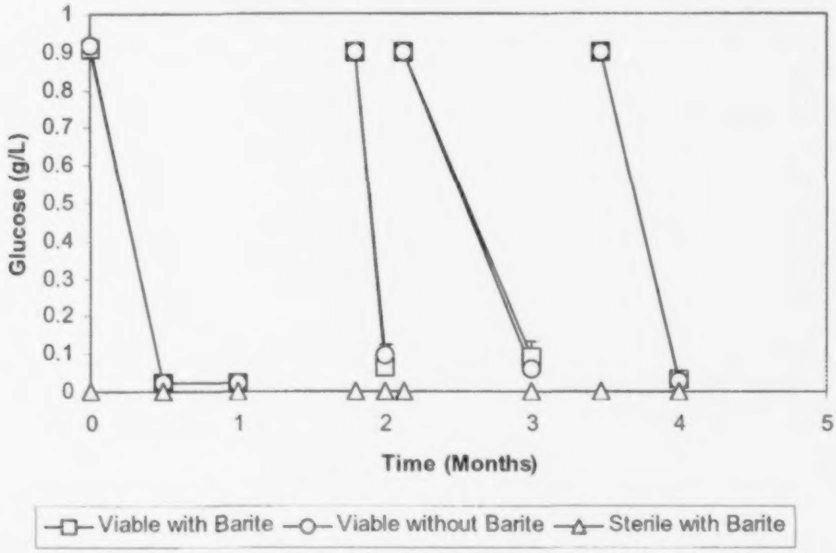

Fig. 4.25. Glucose concentrations in sewage sludge-supplemented microcosms. Glucose concentrations immediately after re-supplementing with glucose at 1.8, 2.2 and 3.6 months were estimated based on the amount of glucose solution added. Error bars show one standard deviation.

There was a considerable amount of variability in the methane production summarized in Fig. 4.26. For example, on day 61, the methane measured in the duplicate microcosms without barite were 14% and 6% (by volume). This variability would be expected because it is difficult to obtain reproducible sub-samples from a soil with a low number of methanogens. However, by day 124 (the last sampling time in Fig. 4.26) there was virtually no difference in the methane concentrations in the duplicate microcosms without barite. After a long incubation period, the maximum methane yield is dependent on the amount of electron donor (glucose) that was supplied, rather than the initial numbers of methanogens in a sub-sample.

Very large error bars were observed with the average methane concentrations obtained from the viable microcosms supplemented with barite (Fig. 4.26). This is because methane production was completely inhibited in two of the triplicate microcosms. As was observed in Fig.

4.23, the addition of barite, a source of sulfate, severely inhibited methanogenesis. This is consistent with the sulfate-reducing bacteria out-competing the methanogens for electron donors. Because of the high rate of methane production in the third microcosm of the triplet set, it is possible that the barite was inadvertently omitted from this microcosm during the preparation and inoculation of the microcosm. However, this was not verified.

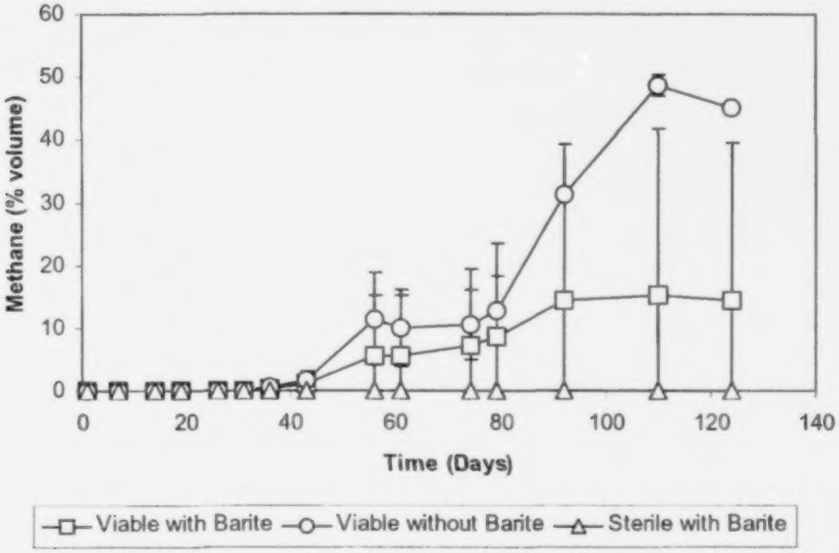

Fig. 4.26 Methane production in the microcosms containing background soil. Error bars are one standard deviation.

Because of the apparent activities of sulfate-reducing bacteria in the barite-supplemented microcosms, slurry samples collected at 3 and 4 months were analyzed for sulfide. After 3 months of incubation the average sulfide concentrations in the sterile, unsupplemented methanogenic microcosms was 2.5±0 μg/g (wet weight) and in the barite-containing viable microcosms was 4.2±0.7 μg/g (wet weight). Based on a t-test, there was no significant difference between these means ($p<0.01$). Similarly, there was no significant difference between the sulfide concentrations measured at 4 months. Thus, despite the strong inhibition of methanogenesis in two of the three microcosms supplemented with barite (Fig. 4.26), the enhanced activity of sulfate-reducing bacteria could not be demonstrated by measuring sulfide production, which was evident in the sulfate-reducing microcosms (Fig. 4.12).

The soluble Ba^{++} detected in the methanogenic microcosms that contained background soil is summarized in Fig. 4.27. Over the 4-month incubation period, there was a general increase in the amounts of soluble Ba^{++} in all of the microcosms. However, there was no statistical difference between the released Ba^{++} in the viable microcosms that received barite and the released Ba^{++} in the sterile controls after 4 months incubation. This is consistent with the results of the sulfide analyses. There was no increase in sulfide concentration in the barite-supplemented viable microcosms, so there should be no increase in released Ba^{++}. Thus, there was no evidence of Ba^{++} release in these methanogenic microcosms.

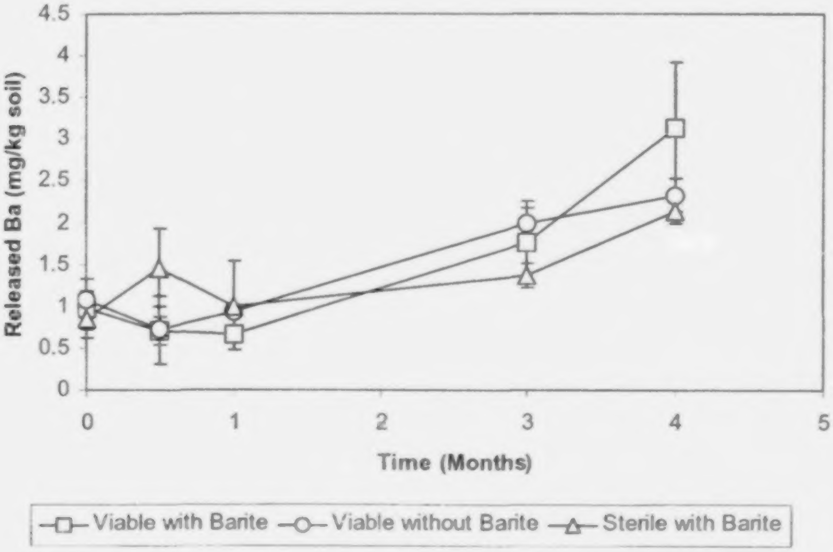

Fig. 4.27. Release of soluble Ba^{++} in the methanogenic microcosms containing background soil. Error bars show one standard deviation.

The results from these microcosms incubated under "methanogenic" suggest that soils which become heavily contaminated with barite may not become methanogenic because of the large supply of sulfate from the barite. This will stimulate the activities of sulfate-reducing bacteria that out-compete methanogens for electron donors.

5 SUMMARY AND CONCLUSIONS

The release of soluble Ba^{++} from barite was expected to be observed in the microcosms incubated under sulfate-reducing conditions. This was observed, indicating that the method used to establish the microcosms and method used for soluble Ba^{++} analysis were reliable. Table 5.1 is an abbreviated overview of the findings of this study. It includes only the results from those microcosms that were supplemented with glucose and 100 mg barite, and did not contain added microorganisms (such as the enrichment of sulfate-reducing bacteria or anaerobic sewage sludge).

Table 5.1 highlights that the release of soluble Ba^{++} from barite was observed in microcosms incubated under aerobic, Mn(IV)-reducing, Fe(III)-reducing and sulfate-reducing conditions. As discussed above, when barite was added to methanogenic microcosms, methanogenesis was inhibited, and sulfate reduction became the predominant microbial process. This would lead to the speculation that soluble Ba^{++} release from barite should have been observed in these microcosms, but it was not (Table 5.1). However, the release of soluble Ba^{++} from barite was observed in the methanogenic microcosms that were supplemented with anaerobic sewage sludge, and the addition of barite stopped methanogenesis (section 4.2.6.1). The reason for the discrepancy between these two sets of methanogenic microcosms is unclear.

The only microcosms in which there was a clear suppression of Ba^{++} dissolution from barite were those incubated under nitrate-reducing conditions. This was likely due to the well documented competition between nitrate-reducing bacteria and sulfate-reducing bacteria.

Table 5.1 also summarizes the maximum Ba^{++} release observed in the various microcosms, and includes ratios that express the extent of the increase in soluble Ba^{++}. Each ratio was calculated by dividing the maximum amount of soluble Ba^{++} in the microcosms by the initial amount of soluble Ba^{++} in the same microcosms. The greatest releases were found in the Fe(III)-reducing and sulfate-reducing microcosms, with values of 14 and 13 mg soluble Ba^{++}/kg soil (wet weight). The highest ratios of released Ba^{++} were in the Mn(IV)-reducing and Fe(III)-reducing microcosms. These showed 36-fold and 41-fold increases, respectively, in the amounts of soluble Ba^{++} under these incubation conditions. Part of the reason for these high ratios is because the initial soluble Ba^{++} concentrations were very low in these two sets of microcosms. The initial soluble Ba^{++} concentration was 31 μg/L in the Mn(IV)-reducing microcosms, and 160 μg/L in the Fe(III)-reducing microcosms. For comparison, the other initial soluble Ba^{++}

concentrations ranged from 230 µg/L in the methanogenic microcosms to 5,100 µg/L in the nitrate-reducing microcosms.

Table 5.1 Summary of the selected parameters from microcosms that contained background soil supplemented with glucose and 100 mg barite. The microbial activities were due to the native microorganisms only (results from microcosms with added microbes are not included).

Incubation condition	Ba^{++} release greater than in sterile control	Maximum Ba^{++} release (mg/kg soil)	Ratio: (Max. Ba^{++}/Time zero Ba^{++})
Aerobic	Yes[a]	8.1	1.7
Nitrate-reducing	No[b]	N/A[d]	N/A
Mn(IV)-reducing	Yes[c]	2.5	36
Fe(III)-reducing	Yes[a]	14	41
Sulfate-reducing	Yes[a]	13	7.6
Methanogenic	No[b]	N/A	N/A

[a] $P<0.01$

[b] No statistical difference between soluble Ba^{++} in the viable cultures and the sterile controls.

[c] $P<0.05$

[d] Not applicable because the amount of soluble Ba^{++} in the viable cultures was not statistically greater than the amount of soluble Ba^{++} in the sterile controls.

The findings of this short-term investigation, using microcosms with ample supplies of glucose as an electron donor suggest that there is a potential for barite dissolution releasing soluble Ba^{++} in soil environments that are dominated by aerobic, Mn(IV)-reducing, Fe(III)-reducing or sulfate-reducing conditions. The rates of microbial release of soluble Ba^{++} in these types of environments would likely be much slower than observed in this study, because the supply of electron donor would be rate limiting in the environment.

6 ACKNOWLEDGMENTS

We are indebted to Dr. G.J. Taylor for allowing us to use his AA spectrophotometer. We thank J. McDonald-Stephens for helping develop the AA procedure for barium analysis and we thank D. Coy for assisting with the preparation of the microcosms and many of the analytical methods. We also acknowledge technical assistance from Jessica Hrudey.

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8 APPENDIX

A.1 Aerobic medium (Sulfate-free – to prevent re-precipitation of $BaSO_4$) Fedorak and Coy (1996)

K_2HPO_4	0.5 g
$NaCl$	2.0 g
$MgCl_2$	0.2 g
KNO_3	2.0 g
NH_4Cl	1.0 g
$FeCl_2 \cdot H_2O$	trace
H_2O	1.0 L
Trace metals	1.0 mL

Trace metals (Fedorak and Grbic´-Galic´, 1991)

$CaCl_2 \cdot 2H_2O$	3.7 g
$ZnCl_2$	0.44 g
H_3BO_3	2.5 g
$MnCl_2$	0.87 g
$FeCl_3$	0.65 g
$Na_2MoO_2 \cdot 2H_2O$	0.29 g
$CoCl_2$	0.01 g
$CuCl_2$	0.0001 g
H_2O	1.0 L

A.2 General procedure for preparing anaerobic media

All media for anaerobic incubations were prepared using a 30% CO_2, 70% N_2 gas mixture. The media can be prepared in two ways:

1. Combine all of the ingredients except $NaHCO_3$ and vitamin solution and boil. Cool under gas mixture. When medium is cool, add $NaHCO_3$ and vitamin solution. Ensure that the pH is near neutral before dispensing into bottles.

2. Combine all of the ingredients. Bubble with gas mixture for at least 30 min before dispensing into bottles. Ensure that the pH is near neutral.

A.3 Preparation of sulfate-free, nitrate-reducing medium

K_2HPO_4	0.636 g
NaCl	2.0 g
NH_4Cl	0.06 g
Trace Metals (solution 2, section A.5)	1.0 mL
Wolfe's Vitamin Solution (section A.4)	1.0 mL
$NaHCO_3$	2.5 g
H_2O	1.0 L

The water was added immediately after it boiled. As the solution cooled, it was sparged with O_2-free gas. Then 100-mL portions were dispensed into ten 158-mL serum bottles that were flushed with O_2-free gas. These were sealed with rubber stoppers and autoclaved.

A solution of 20 mg/mL $CaCl_2$ and 20 mg/mL $MgCl_2$ was prepared by mixing the following in a serum bottle:

$CaCl_2 \cdot 2H_2O$	2.7 g
$MgCl_2 \cdot 6H_2O$	4.3 g
H_2O (Freshly boiled)	100 mL

This bottle was sealed with a rubber stopper and autoclaved.

After autoclaving, a 1-mL portion of the $MgCl_2$ and $CaCl_2$ solution was dispensed (using a syringe) into each bottle of nitrate-reducing medium.

A.4 Mn(IV) and Fe(III) reduction: (Lovley and Phillips, 1986, 1988)

$NaHCO_3$	2.5 g
$CaCl_2 \cdot H_2O$	0.1 g
KCl	0.1 g
NH_4Cl	0.06 g
$NaH_2PO_4 \cdot H_2O$	0.6 g
Wolfe's Vitamin Solution	1.0 mL
Wolfe's Mineral Solution	1.0 mL
H_2O	1.0 L

Slurry of MnO_2 or $Fe(OH)_3$*

*For Fe(III)-reducing medium, the $Fe(OH)_3$ slurry was prepared as outlined by Lovley and Phillips (1986). For the Mn(IV)-reducing medium, the MnO_2 slurry was prepared as outlined by Lovley and Phillips (1988). Fifteen millilitres of the appropriate slurry was added to 85 mL of medium.

Wolfe's Vitamin Solution
(ATCC, 1992)

Biotin	2.0 mg
Folic acid	2.0 mg
Pyroxidine-HCl	10.0 mg
Thiamine-HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
Na Pantothenate	5.0 mg
Cyanocobalamine	trace
p-Aminobenzoate	5.0 mg
Thioctic acid	5.0 mg
H_2O	100 mL

Wolfe's Mineral Solution
(ATCC, 1992)

Nitrilotriacetic acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoCl_2 \cdot 6H_2O$	0.1 g
$CaCl_2$	0.1 g
$ZnSO_4 \cdot 7H_2O$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
$NiCl_2 \cdot 6H_2O$	0.025 g
H_2O	100 mL

A.5 Sulfate reduction: – Sulfate-free, $BaSO_4$ is the source of sulfate.
(Widdel-Pfennig medium - freshwater composition, Collins and Widdel, 1986)

Solution 1 (modified)	960 mL
Solution 2	1 mL
Solution 3	1 mL
Solution 4	30 mL
Solution 5	1 mL
Solution 6	1 mL

Solution 1 (Modified to be sulfate-free)

NaCl	1.0 g
$MgCl_2 \cdot 6H_2O$	0.4 g
NH_4Cl	0.1 g
KH_2PO_4	0.2 g
KCl	0.3 g
$CaCl_2 \cdot 2H_2O$	0.15 g
Resazurin (0.01 g/100 mL H_2O)	10 mL
H_2O	960 mL

Autoclave

Solution 2

HCl	4.5 mL
$FeCl_2 \cdot 4H_2O$	1.5 g
H_3BO_3	60 mg
$MnCl_2 \cdot 4H_2O$	100 mg
$CoCl_2 \cdot 6H_2O$	120 mg
$ZnCl_2$	70 mg
$NiCl_2 \cdot 6H_2O$	25 mg
$CuCl_2 \cdot 2H_2O$	15 mg
$Na_2MoO_4 \cdot 2H_2O$	25 mg
H_2O	995 mL

Autoclave

Solution 3

$Na_2SeO_3 \cdot 5H_2O$	3 mg
NaOH	0.5 g
H_2O	1.0 L

Autoclave

Solution 4

$NaHCO_3$	8.4 g
H_2O	100 mL

Saturate with CO_2, then filter sterilize

Solution 5

$Na_2S \cdot 9H_2O$	1.2 g
H_2O	10 mL

Boil H_2O

Solution 6

Biotin	10 mg
4-Aminobenzoic acid	40 mg
Thiamine-HCl	100 mg
B12	50 mg
Nicotinic acid	100 mg
H_2O	1.0 L

Filter sterilize

A.6 Methanogenic Medium (Fedorak and Hrudey, 1984)

Mineral Solution I	1.0 mL
Mineral Solution II	0.1 mL
Resazurin Solution	1.0 mL
Phosphate Solution	1.0 mL
$NaHCO_3$	0.57 g
H_2O	100 mL

Mineral Solution I

NaCl	5.0 g
$CaCl_2 \cdot H_2O$	1.0 g
NH_4Cl	1.0 g
$MgCl_2 \cdot 6H_2O$	1.0 g
0.01 M HCl	100 mL

Mineral Solution II

$(NH_4)_6Mo_7O_{24} \cdot 2H_2O$	1.0 g
$ZnSO_4 \cdot 7H_2O$	0.01 g
H_3BO_3	0.03 g
$FeCl_2 \cdot 4H_2O$	0.15 g
$CoCl_2 \cdot 6H_2O$	1.0 g
$MnCl_2 \cdot 4H_2O$	0.003 g
$NiCl_2 \cdot 6H_2O$	0.003 g
$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g
H_2O	100 mL

Phosphate Solution

5.0 g KH_2PO_4 per 100 mL H_2O

Resazurin Solution

0.01 g per 100 mL H_2O

APPENDIX II

BARIUM MOBILIZATION BY SALT

APPENDIX II – BARIUM MOBILIZATION BY SALT

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1. INTRODUCTION

As part of the project to develop soil quality guidelines for barite, consideration was given to various conditions that could result in the mobilization of barium from barite. One of the conditions considered was the presence of high concentrations of salts, since releases of oilfield brine may have occurred in areas where oilfield barite is present. Typical oilfield brines are predominantly sodium chloride.

2. STUDY OBJECTIVE

The objective of this study was to determine whether the addition of sodium chloride to soil could release barium either from the soil or from barite added to the soil. It was intended that the range of salt concentrations used should be consistent with the range of electrical conductivities (ECs) typically found in soil at former oilfield facilities where brine releases have occurred (*i.e.*, up to 20 dS/m).

3. MATERIALS AND METHODS

3.1 Range Finding Experiment

Sixteen microcosms were set up as a range finding experiment to determine the relation between the amount of NaCl added and the EC of the supernatant. These sixteen microcosms contained approximately 50 g of soil (eight with contaminated soil and eight with background soil) and 100 mL of double distilled H_2O. 0 mg, 200 mg, 400 mg, 600 mg, 800 mg, 1,000 mg, 1,200 mg, and 1,400 mg NaCl were added to the eight flasks of background soil and this process was repeated for the contaminated soil. The flasks were covered in parafilm (to avoid evaporation) and placed on a shaker to shake for approximately 24 hours. After 24 hours, a small sample of each microcosm was placed in an Eppendorf Centrifuge tube and the sixteen tubes were centrifuged. Then the EC of the supernatant from these samples was measured. These results were used to generate standard curves (Figure II-1) for contaminated soil and for background soil to determine the mass of NaCl necessary to produce electrical conductivities of 0 dS/m, 2 dS/m, 4 dS/m, 8 dS/m and 20 dS/m.

3.2 Quantitative Experiment

Once the necessary amount of salt was determined to achieve the above-noted EC values, 20 microcosms were prepared: five microcosms contained approximately 50 g of background soil, 100 mL of double distilled H_2O and the respective amount of NaCl to produce the above-noted ECs; five microcosms were identical to the previous five, but each also contained approximately 100 mg of barite; five microcosms were identical to the first five, but each also contained 500 mg of barite; and five microcosms contained approximately 50 g contaminated soil, 100 mL of double distilled H_2O and the respective amounts of NaCl needed to generate the desired ECs.

These twenty microcosms were sealed with parafilm and placed on a shaker to shake for approximately 24 hours. After twenty-four hours 13 mL was removed from each sample and placed in large centrifuge tube and centrifuged for ten minutes. 1.4 mL of supernatant was transferred to Eppendorf tubes and stored for barium analysis. The remaining supernatant was used to measure the EC of each of the 20 microcosms.

4. RESULTS

The results of the quantitative experiment are summarized in Table II-1 and Figure II-2.

5. CONCLUSIONS

The conclusions of this experiment are evident from Figure II-2 and are that adding salt to barite-contaminated soil can result in an increase in the soluble barium present, but that the barium released comes from the soil, and not from the barite. Accordingly, this process will not be significant when determining how much barite can safely remain in soil.

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Table II-1. Barium Mobilization Data

	Mass of NaCl Added (mg)	Mass of Soil (g)	Mass of Barite Added (mg)	Measured EC (dS/m)	Soluble Ba^{2+} (mg/L)
Background Soil	0	50.15	0	0.22	0.02
	90.1	50.44	0	1.76	0.19
	232.9	50.13	0	3.7	0.41
	518.5	50.19	0	8.3	1
	1,375.70	50.17	0	19.6	3.4
Background + 2,000 mg/kg Barite	0	50.43	100.8	0.24	0.12
	90.1	50.52	99.9	1.74	0.23
	232.7	50.41	101.4	3.9	0.45
	518.7	50.21	101	8.3	0.96
	1,375.70	50.42	99.9	19.8	3.3
Background + 10,000 mg/kg Barite	0	50.54	499.8	0.26	0.11
	90.1	50.21	500.4	1.75	0.24
	232.9	50.56	500.7	3.9	0.65
	518.6	50.4	500	8.3	1
	1,375.60	50.02	500.4	19.7	2.9
Contaminated Soil	0	50.38	0	0.6	0.16
	64	50.6	0	1.64	0.11
	208.2	50.54	0	4.1	0.2
	496.1	50.2	0	8.4	0.37
	1,354.40	50.61	0	19.2	1

Figure II-1. Range Finding Test

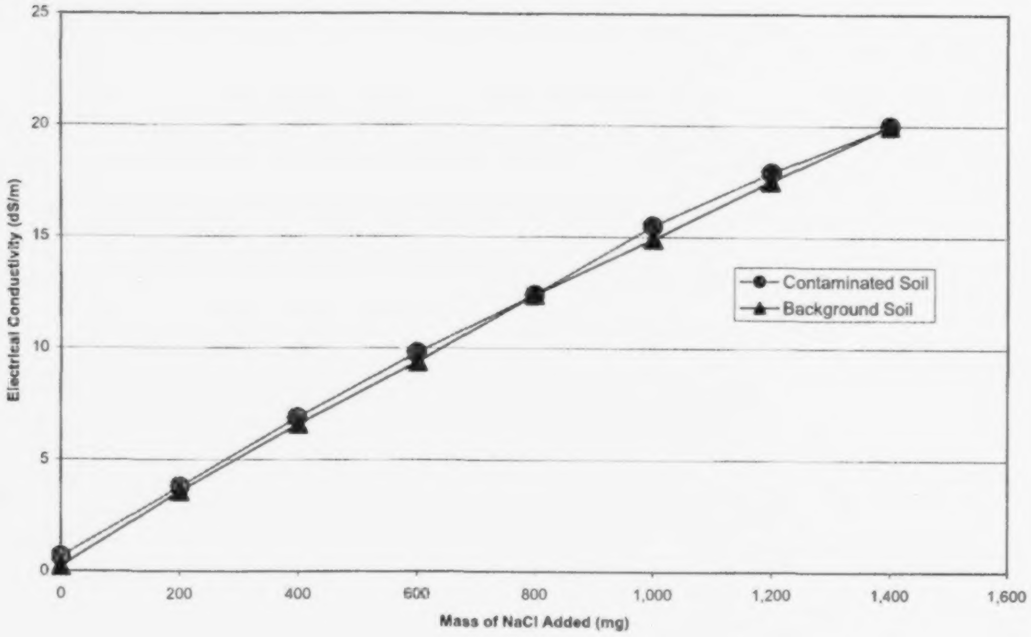
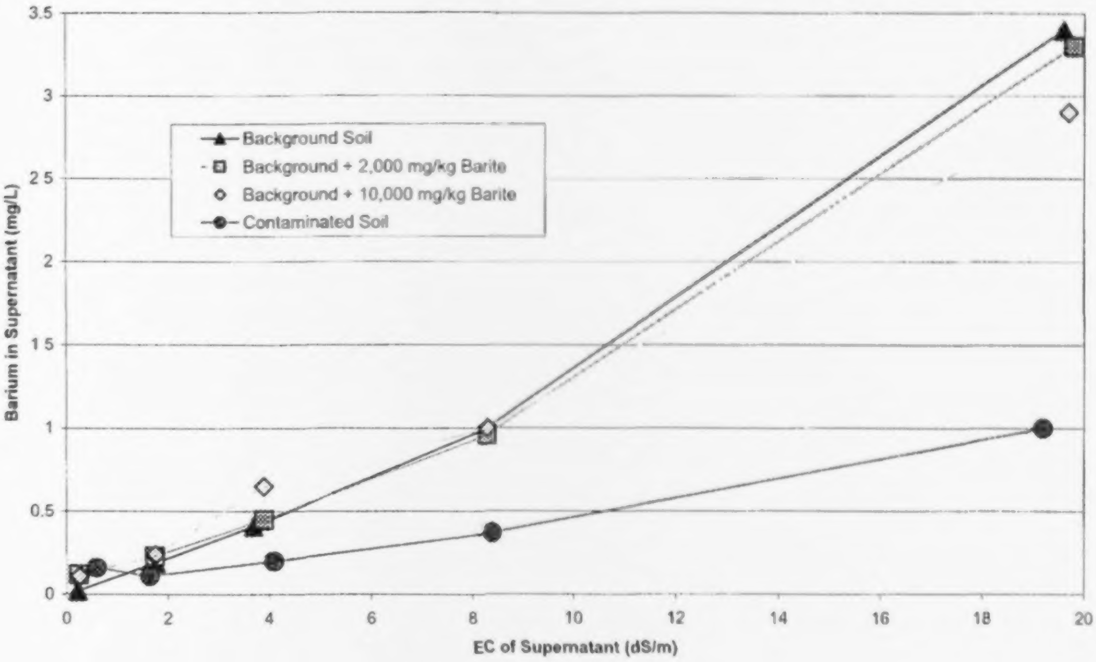


Figure II-2. Mobilization of Barium by Addition of NaCl



APPENDIX III

ESG (2003A)

ECOTOXICITY EVALUATION OF REFERENCE SITE SOILS AMENDED WITH BARIUM SULPHATE

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APPENDICES

Appendix A Results of the Amended Site Soil Assessment: Ecotoxicity Evaluation with Plant and Invertebrate Species

1. INTRODUCTION

In February 2002, ESG International was contracted by Komex International, Ltd. to provide an ecotoxicity assessment of site soils contaminated with a mixture of petroleum hydrocarbons and barium sulphate. The soils originated from an abandoned wellsite location near Pincher Creek, Alberta, and the site had been contaminated as a consequence of drilling activities (i.e., drilling mud disposal). The results of the acute earthworm test indicated that the contaminated site soil was not acutely toxic to earthworms; however, there was a 28 to 72% reduction in growth of two of the three plant species tested, following longer-term exposure to the contaminated soil, relative to the uncontaminated reference control soil. The barium sulphate soil concentration, as total barium sulphate, was at levels that exceeded federal (CCME) and Alberta provincial (Tier 1 - 1994) guideline criteria; however, the level of barium sulphate as saturated paste, which represents the water-soluble fraction, was extremely low. Petroleum hydrocarbon soil concentrations in the contaminated soil were at levels that exceeded the regulatory guideline criteria (CCME Fractions 2 and 3) and might have caused the adverse effects. As a result, Komex International contracted ESG to provide an ecotoxicity assessment of the previously collected reference control site soil amended with barium sulphate. The uncontaminated reference site soil is a very clayey soil collected by Komex International from a site in Alberta. The physical and chemical characteristics of the reference control soil were similar to those of the contaminated site soil, but free of contaminants.

A preliminary toxicity assessment (Phase 1) with barium sulphate-amended reference soil was conducted. It consisted of an acute earthworm toxicity test and an acute plant screening test. The test species were the earthworm, *Eisenia andrei*, and the plant alsike clover (*Trifolium hybridum*). The results of these preliminary tests were reported earlier (ESG 2002); they are also included in this report. A more comprehensive testing program (Phase 2) with higher barium sulphate soil concentrations was subsequently conducted and consisted of one acute earthworm test, one acute arthropod (springtail) test and three definitive plant tests. All experiments were conducted with undiluted background soil (e.g. a field-collected uncontaminated reference soil) amended with different quantities of industrial-grade barium sulphate, as well as with an experimental control soil (e.g. artificial soil). The earthworm and plant test species were identical to those used in the previous testing program with the contaminated site soil. The test species included the earthworm, *Eisenia andrei* (compost worm), the leguminous plant, alsike clover (*Trifolium hybridum*), and the monocotyledonous perennial ryegrass (*Lolium perenne*), and orchardgrass (*Dactylis glomerata*). These species had been originally chosen because *E. andrei* is commonly used to assess the effects of contaminated soil on terrestrial invertebrates, and the three plant species are used as forage crops on rangeland in Alberta. The arthropod species, a springtail, *Onychiurus folsomi*, was also included because it inhabits a niche in the terrestrial ecosystem different from that of earthworms and plants and it represents a different exposure pathway.

The soils used were those previously collected by Komex International and received by ESG on February 27, 2002. After their arrival at ESG's Southgate Laboratory, the soil samples (e.g., 20-L buckets) had been assigned identification numbers and were entered into a log book (Table A.1; Appendix A). Six buckets of the background soil had been received, and the samples were denoted as Background (1-6). Prior to testing, the soil samples had been air-dried for 24 hours, broken up by hand, pulverized into smaller aggregates, and sieved through a 6-mm stainless steel sieve. Once all the soil from the six buckets had been sieved, they were homogenized and were stored in plastic 20-L buckets at room temperature (20 ± 2°C) until use. The experimental control soil was an artificial soil (AS) formulated in the laboratory, and characterized as a fine sandy loam. The soil moisture contents of the background and artificial soils were determined prior to testing. The physical and chemical characteristics of the experimental control soil and the reference (background) control soil are listed in Table A.2 (Appendix A).

The objective of this research was to determine the concentration of barium sulphate in the reference control soil (background soil) that would cause an adverse effect on earthworm survival, springtail survival and seedling emergence and early growth. The experimental control soil provided QA/QC data on test organism health and experimental conditions. Preliminary testing began on July 30, 2002, and the more comprehensive testing program began on September 24, 2002.

2. PHASE 1: ACUTE TOXICITY TO *EISENIA ANDREI*

2.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 500, 1500, 5000, 10,000, 25,000, and 50,000 mg $BaSO_4$/kg soil dry weight (d.w.).

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 (AS) or 350 (background reference soil) g test soil wet weight (w.w.). Mass of soil per test unit varied depending on the bulk density of the soil. Jars were covered with perforated tin foil held by a metal screw ring to facilitate air exchange for the earthworms. Each treatment had 3 replicate test units, each with five adult earthworms. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

2.2 Soil Preparation

On Day -1, the control and amended soils were prepared on a dry weight basis, but the volumes were adjusted to compensate for differences in the bulk density of the various soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 13 to 37%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.3; Appendix A).

On Day 0, five clitellate *Eisenia andrei* were added to each test unit and the soils in the test units were misted gently with deionised water as necessary. Substantially more water was added to the test units of the reference soil treatments after the earthworms were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of deionised water while homogenizing the soil; therefore most of the water was added when the earthworms were added.

2.3 Statistical Analyses

No statistical analyses of the earthworm toxicity data were conducted because no mortality was observed. The data were tabulated instead.

2.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei*. All earthworms in each treatment survived (n = 15), and all surviving worms appeared healthy (Table A.4; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei*. No mortality in any of the treatments occurred following 14 days of exposure, and all surviving worms appeared healthy (Table A.4; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for *E. andrei* survival and growth (Table A.3; Appendix A).

3. PHASE 1: ACUTE SCREENING ALSIKE CLOVER TEST

3.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d;
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 400 einsteins/m^2;
- Test endpoint: seedling emergence, shoot and root wet and dry mass.

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 500, 1500, 5000, 10,000, 25,000, and 50,000 mg $BaSO_4$/kg soil dry weight (d.w.).

Test units consisted of a glass petri dish enclosed within an inverted 1-L clear polypropylene container. Each petri dish was filled with either 70 g w.w. of AS at 35% moisture content or 100 g w.w. of reference ("Background") soil at 18% moisture content. More soil was added to test units containing the reference soil to compensate for differences in bulk densities between the soils. Both AS and reference soil treatments consisted of two replicate test units. Ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

3.2 Soil Preparation

On Day 0, the control and amended soils were prepared on a dry weight basis, but volumes were adjusted to compensate for differences in bulk density of the soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 13 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of deionised water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.3; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with deionised water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked daily and watered as necessary with well-aerated de-chlorinated, municipal tap water.

3.3 Test Processing

On Day 14, each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than three mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment was recorded.

3.4 Statistical Analyses

Analysis of variance procedures were applied to the data followed by Dunnett's 2-sided pairwise comparison tests to compare treatment means of the amended reference soils to the control reference soil. To ensure that the assumptions of the analytical tests performed were met, the data and residuals were examined for normality and for homogeneity of variance among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997).

3.5 Results

Alsike clover emergence in the reference soil was not significantly affected by exposure to barium sulphate up to concentrations of 50,000 mg $BaSO_4$/kg soil d.w. (Figure A.1; Appendix A).

In general seedling growth also was not significantly affected by exposure to barium sulphate up to concentrations of 50,000 mg $BaSO_4$/kg soil d.w. Only one of the six endpoints (root length) was significantly lower than the root length of plants in the reference control soil (0 mg $BaSO_4$/kg soil d.w.) (Table A.5, Figure A.2; Appendix A), at the highest barium sulphate concentration tested (50,000 mg $BaSO_4$/kg soil d.w.). There were no obvious effects of exposure to barium sulphate on the abundance of nodules observed per plant per treatment.

Soil pH and electrical conductivity were measured and compared among the test soils, and the values for both parameters in all soil treatments were within the range that supports plant growth. The addition of barium sulphate to the reference soil did not affect pH or conductivity (Table A.3; Appendix A).

4. PHASE 2: CHEMICAL ANALYSES OF THE AMENDED SOIL

Prior to testing (Day 0), and at the end of testing (Day 14), the barium soil concentration of the BaSO4-amended soils was measured. Soil subsamples were collected from each spiked treatment (0 to 1,000,000 mg BaSO4/kg soil d.w.) at the beginning and end of the *Eisenia andrei* and perennial ryegrass Phase 2 tests. Samples were collected from one earthworm and one plant test to observe the effect, if any, of the presence of earthworms or plants on the total or water-soluble concentration of barium in the soil. Barium sulphate was analysed as total soil concentration and as saturated paste. Barium sulphate is sparingly soluble and, as a result, the $BaSO_4$-saturated paste values represent the water-soluble fraction of barium sulphate in the soil. The chemical analyses were performed by Philip Analytical Services Corporation, Mississauga, Ontario.

The total barium and barium as saturated paste concentrations in the soil samples collected from the *Eisenia andrei* and perennial ryegrass tests are presented in Table A.6 (Appendix A). There were no notable differences in any of the measured soil concentrations between the *E. andrei* and perennial ryegrass samples.

The reference control soil (0 mg/kg) had relatively high background total barium concentrations that ranged from 273 to 1160 ppm. Total barium soil concentrations in the lowest (1000 mg/kg) treatments ranged from 714 to 791 ppm. The measured concentrations are in general in agreement with the nominal concentration once background barium concentrations are accounted for, since 1000 g barium sulphate is equivalent to 588.4 g of barium. The total barium soil concentrations for all of the other nominal treatments however, were one to three orders of magnitude lower than expected. No concentration-response pattern was evident, and regardless of sampling time or source, the concentration of total measured barium was lowest at the highest nominal concentration (1,000,000 mg $BaSO_4$/kg soil d.w., or 100% $BaSO_4$). Despite this, the results were reasonably consistent; total soil concentrations were similar among the 10,000 to 1,000,000 mg/kg treatments, and ranged from 1060 to 1990 ppm. Within a nominal concentration, measured total barium concentrations were even more similar, particularly within the same sampling day. The analytical procedure used to measure total barium soil concentration was the Ontario Ministry of the Environment (OMOE) protocol E3073A, which is an aqua regia digest. OMOE protocol E3073A is the standard protocol used in Ontario to measure total soil metal concentrations. An aqua regia digest is an aggressive extraction procedure that uses hydrochloric and nitric acid to leach contaminants of interest from a sample. It is a standard extraction process used to extract inorganic contaminants, particularly metals, from soils. However, barium as the barium sulphate salt is not soluble in acid, therefore this particular method of extracting total barium from the soil samples was likely inappropriate.

The concentration of $BaSO_4$-saturated paste at Day 0 was below the detection limit (0.05 mg/L) in all samples from both tests, except for the highest concentration where it was still very low (0.11 to 0.30 mg/L). Barium was also detected (0.07 mg/L) in the control sample from the *E. andrei* test. Barium (as saturated paste) levels were higher in all the Day 14 samples and appeared to follow a consistent concentration–response pattern. The concentrations were still low, however, and ranged from 0.056 to 2.40 mg/L (Table A.6). This is consistent with the sparingly soluble nature of barium sulphate and suggests that it might be only slightly bioavailable to exposed soil organisms.

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5. PHASE 2: ACUTE TOXICITY TO *EISENIA ANDREI*

5.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 (AS) to 350 (background reference soil) g soil wet weight (w.w.) of test soil. Mass of soil added per test unit varied depending on the bulk density of the soil. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 3 replicate test units. Five adult earthworms were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

5.2 Soil Preparation

The test soils were prepared as described in Subsection 2.2.

5.3 Statistical Analyses

No statistical analyses of the earthworm toxicity data were conducted because there was no observed mortality; the results were simply tabulated.

5.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei*. All earthworms in each treatment survived (n = 15), and all surviving worms appeared healthy (Table A.4; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei*. No mortality in any of the treatments occurred following 14 days of exposure, and all surviving worms appeared healthy (Table A.4; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil increased soil pH however, all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) decreased with increasing concentrations of barium sulphate, however the soil moisture in all treatments was adequate for *E. andrei* survival and growth. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for *E. andrei* survival and growth (Table A.3; Appendix A).

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6. PHASE 2: ACUTE TOXICITY TO *ONYCHIURUS FOLSOMI*

6.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 7-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-hr day, 8-hr night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of 125-mL, wide-mouthed glass mason jars filled with 35 g soil wet weight (w.w.) of test soil. Jars were closed loosely with metal lids held by a metal screw ring to prevent escape of the organisms. Each treatment included 3 replicate test units. Ten mature springtails were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998c).

6.2 Soil Preparation

On Day -1, the control and amended soils were prepared on a dry weight basis, but the volumes were adjusted to compensate for differences in the bulk density of the various soils. Barium sulphate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $BaSO_4$-soil mixture was then hydrated with deionised water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 8 to 33%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.3; Appendix A).

On Day 0, ten mature *Onychiurus folsomi* were added to each test unit and the soils in the test units were misted gently with deionised water as necessary. More water was added to the test units of the reference soil treatments after the springtails were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of deionised water while homogenizing the soil therefore most of the water was added when the springtails were added.

6.3 Statistical Analyses

No statistical analyses of the springtail toxicity data were conducted because there was no observed mortality; the data were simply tabulated.

6.4 Results

On Day 7, each test unit was inspected to determine the number and condition of surviving *O. folsomi*. All springtails in each treatment survived (n = 30), and all surviving springtails, as far as it was ascertainable, appeared healthy (Table A.7; Appendix A).

Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg $BaSO_4$/kg soil d.w.), and $BaSO_4$-amended reference soils. The addition of barium sulphate to the reference soil increased soil pH however all soil pH values were within a range acceptable for O. *folsomi* survival and growth. Soil moisture content (end of test) decreased with increasing concentrations of barium sulphate, however the soil moisture in all treatments was adequate for O. *folsomi* survival. Soil electrical conductivity in the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity in all soil treatments was well within the range considered acceptable for O. *folsomi* survival (Table A.3; Appendix A).

7. PHASE 2: DEFINITIVE PLANT TOXICITY TESTS

7.1 Experimental Design and Conditions

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover, orchardgrass and perennial ryegrass from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d (orchardgrass and perennial ryegrass), 21 d (alsike clover);
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 400 einsteins/m^2;
- Test endpoint: seedling emergence, shoot and root wet and dry mass and, number of nodules per plant (alsike clover only).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background") soil, and batches of reference ("Background") soil amended with sufficient barium sulphate to achieve the following concentration series; 1000, 10,000, 30,000, 100,000, 300,000, and 1,000,000 mg $BaSO_4$/kg soil d.w.

Test units consisted of a 1-L clear polypropylene container. Each container was filled with either 485 g w.w. of AS at 35% moisture content or 375 g w.w. of reference ("Background") soil at 18% moisture content. More soil was added to test units containing the reference soil to compensate for differences in bulk densities between the soils. Both AS and reference soil treatments consisted of 4 replicate test units. Five orchardgrass and perennial ryegrass seeds, and ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

7.2 Soil Preparation

Test soils were prepared as described in Subsection 3.2..

7.3 Test Processing

The test units were processed on Day 14 or 21 as described in Subsection 3.3. but, in addition, the roots of alsike clover plants were inspected after length measurements were taken, and the number of nodules present per plant was recorded.

7.4 Statistical Analyses

Analyses of variance procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil (SPSS, 1997). Where the data was amenable, linear or non-linear regression analyses were also conducted in order to generate IC50 and IC20 estimates. The analyses consisted of using a linear or four nonlinear regression models (i.e. logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variance among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998b) or Stephenson *et al.* (2000).

7.5 Results

7.5.1 Alsike clover

Alsike clover emergence in the reference soil was significantly decreased by exposure to barium sulphate at the highest concentration only (1,000,000 mg $BaSO_4$/kg soil d.w., or 100% $BaSO_4$) (Figure A.3; Appendix A). Pure (100%) barium sulphate is a very dense growth matrix for plants, and it is possible that the decreased emergence of alsike clover in this treatment was the result of a physical inhibition of emergence, rather than from chemical toxicity.

Barium sulphate in the reference soil in general did not adversely affect alsike clover seedling growth. Where there was a significant difference, seedling growth was greater in plants exposed to the barium sulphate-amended soils (Figures A.4 and A.5, Table A.9; Appendix A). Shoot length at the highest treatment concentration (1,000,000 mg $BaSO_4$/kg soil d.w.) was significantly lower relative to that in the control treatment. Of the six growth endpoints, only shoot dry mass data were amenable to regression analysis. Linear regression analysis of the shoot dry mass data estimated IC50 and IC20s greater than 1,000,000 mg/kg (100% barium sulphate) (Table A.8; Appendix A). Nodule production of alsike clover roots was not significantly affected by exposure to barium sulphate-amended soils (Figures A.6 and A.7; Appendix A).

7.5.2 Orchardgrass

Orchardgrass seedling emergence in the reference soil was not significantly affected by exposure to barium sulphate at concentrations up to 1,000,000 mg $BaSO_4$/kg soil d.w. (Figure A.3; Appendix A).

Barium sulphate in the reference soil did not adversely affect orchardgrass seedling growth. Where there was a significant difference in growth compared to the control treatment, seedling growth was greater in the barium sulphate-amended soils (Figures A.8 and A.9, Table A.9; Appendix A). Of the six growth endpoints measured, only root length data were amenable to regression analysis. Linear regression analysis of the root length data estimated IC50 and IC20s of > 1,000,000 and 850,000 mg/kg, respectively (Table A.8). Of all the endpoints, only root length appeared to be somewhat affected.

7.5.3 Perennial ryegrass

Perennial ryegrass seedling emergence in the reference soil was not significantly affected by exposure to barium sulphate at concentrations up to 1,000,000 mg $BaSO_4$/kg soil d.w. (Figure A.3; Appendix A).

Barium sulphate in the reference soil in general did not adversely affect perennial ryegrass seedling growth. Shoot growth was significantly greater in the reference soil amended with 1,000,000 mg $BaSO_4$/kg soil d.w. (Figures A.10 and A.11, Table A.9; Appendix A). Root length was significantly decreased following exposure to barium sulphate-amended soils only at the highest treatment concentration (1,000,000 mg $BaSO_4$/kg soil d.w.). Of the six growth endpoints measured, only root length data were amenable to regression analysis. Non-linear regression analyses of the root length data estimated IC50 and IC20s of >1,000,000 and 960,000 mg/kg, respectively (Table A.8; Appendix A).

7.5.4 Soil physical and chemical characteristics

As with the earthworm and collembola Phase 2 test soils, the addition of barium sulphate to the reference soil in all three plant tests increased soil pH, however all soil pH values were within the range that supports plant growth. Soil moisture content (beginning of test) decreased with increasing concentrations of barium sulphate; however water was added to increase moisture levels to close to saturation after the seeds were planted. Electrical conductivity of the reference soil was not affected by the addition of barium sulphate, and the electrical conductivity of all soil treatments was well within the range that supports plant growth (Table A.3; Appendix A).

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8. DISCUSSION

The results from both the Phase 1 and Phase 2 tests suggest that barium sulphate is not acutely toxic to the terrestrial invertebrates, even at nominal concentrations as high as 1,000,000 mg $BaSO_4$/kg soil d.w. Barium sulphate was not acutely toxic to alsike clover up to 50,000 mg $BaSO_4$/kg soil d.w.. The results from the Phase 2 definitive toxicity testing with alsike clover, orchardgrass, and perennial ryegrass indicate that longer-term exposure to barium sulphate did not result in toxicity to the three plant species, even at the highest exposure concentrations possible (100% $BaSO_4$ or 1,000,000 mg/kg soil d.w.). The amendment of barium sulphate to the reference control soil did not substantially alter the physical and chemical characteristics of the soil.

Natalie

Natalie Feisthauer, Project Manager

Gladys I Stephenson

Gladys Stephenson, Project Director

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APPENDIX A

RESULTS OF THE AMENDED SITE SOIL ASSESSMENT: ECOTOXICITY EVALUATION WITH PLANT AND INVERTEBRATE SPECIES

Table A.1. Identification and description of the Alberta reference site soils received from Komex International.

Sample	Date Received	Assigned Sample Identification	Observations
Background Reference Soil	27/02/2002	G2144 Background-1	Light yellow/grey clay, extremely chunky, and moist to dry.
Background Reference Soil	27/02/2002	G2144 Background-2	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-3	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-4	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-5	Same as G2144 Background-1
Background Reference Soil	27/02/2002	G2144 Background-6	Same as G2144 Background-1

Table A.2. Physico-chemical characteristics of the experimental control soil (artificial soil, AS).

Parameter	Artificial Soil (AS)	Background Reference Soil (RS)	Analytical Method
Phosphorous (mg/kg)	23	< 1	Nitric/perchloric acid digestion
Potassium (mg/kg)	22	194	NH_4Ac extractable
Magnesium (mg/kg)	149	846	NH_4Ac extractable
Calcium (mg/kg)	1848	4026	NH_4Ac extractable
Sodium (mg/kg)	67	73	NH_4Ac extractable
Total Carbon (%)	4.46	1.23	Leco furnace method
Total Nitrogen (%)	0.05	0.07	Kjeldahl method
C.E.C. (Cmol+/kg)	18.5	23.0	Barium chloride method
Soil Texture	Fine Sandy Loam	Clay Loam	Gravimetric grain size distribution
Sand (%)	77.3	29.9	
Silt (%)	7.8	40.7	
Clay (%)	14.9	29.5	
Organic Matter (%)	9	1.2	Dichromate oxidation
Bulk Density (g/cm^3)	0.98	1.28	Clod method
pH (units)	6.09	7.7	Water method (1:2)
Conductivity (mS/cm)	0.3	0.54	Saturated paste method
Soil Moisture (%) at Field Capacity (0.3 bar)	N/A	24.4	Gravimetric analysis
Source	Formulated from constituents	Alberta	

C.E.C Cation Exchange Capacity

Table A.3. Physico-chemical characteristics of treatment soils (artificial soil, reference control and amended site soils) from Phase 1 and 2 testing, following test preparation and processing.

Test	Treatment (mg $BaSO_4$/kg reference soil d.w.)	Initial pH[1]	Final pH[2]	Initial Conductivity (µS/m)	Final Conductivity (µS/cm)	Initial Moisture Content (%)	Final Moisture Content (%)
Phase 1 Testing							
Eisenia andrei	Artificial Soil	6.57	5.92	144	166	37.1	36.9
	Reference Soil	7.90	7.84	201	188	15.1*	24.5
	500	7.98	7.90	203	203	12.8*	23.1
	1500	8.02	7.96	214	207	12.9*	22.6
	5000	8.03	7.99	223	212	15.1*	22.6
	10,000	8.01	8.02	215	202	15.4*	23.5
	25,000	7.98	7.99	224	211	15.0*	23.3
	50,000	7.99	8.04	235	215	14.3*	22.2
Alsike Clover	Artificial Soil	6.57	7.45	144	214	[3]	[3]
	Reference Soil	7.90	8.26	201	231	[3]	[3]
	500	7.98	8.33	203	216	[3]	[3]
	1500	8.02	8.29	214	238	[3]	[3]
	5000	8.03	8.32	223	224	[3]	[3]
	10,000	8.01	8.33	215	246	[3]	[3]
	25,000	7.98	8.31	224	275	[3]	[3]
	50,000	7.99	8.36	235	233	[3]	[3]
Phase 2 Testing							
E. andrei	Artificial Soil	6.91	6.76	192	203	32.7	35.6
	Reference Soil	7.93	8.08	204	200	14.1*	20.6
	1000	8.03	8.19	206	204	15.3*	20.9
	10000	8.03	8.15	216	215	13.6*	20.3
	30000	8.01	8.23	220	223	13.7*	19.2
	100000	8.04	8.27	220	222	14.0*	19.3
	300000	8.08	8.28	228	226	13.2*	18.9
	1000000	8.39	8.48	220	187	8.0*	10.8
O. folsomi	Artificial Soil	6.91	6.48	192	219	32.7	32.6
	Reference Soil	7.93	7.85	204	208	14.1*	17.3
	1000	8.03	7.8	206	220	15.3*	18.7
	10000	8.03	7.87	216	221	13.6*	18.5
	30000	8.01	7.85	220	225	13.7*	17.7
	100000	8.04	8.03	220	219	14.0*	14.7
	300000	8.08	8.04	228	218	13.2*	16.2
	1000000	8.39	8.21	220	212	8.0*	9.8
Alsike Clover	Artificial Soil	7.54	7.97	221	436	31.9	[3]
	Reference Soil	7.94	8.02	193	480	15.8	[3]
	1000	7.98	8.12	214	402	13.1	[3]
	10000	7.99	8.17	228	464	15.9	[3]
	30000	8	8.25	219	428	15.6	[3]
	100000	7.99	8.18	222	478	14.8	[3]
	300000	8.01	8.32	230	490	13.4	[3]
	1000000	8.3	9.06	220	439	8.2	[3]

Table A.3. Physico-chemical characteristics of treatment soils (artificial soil, reference control and amended site soils) from Phase 1 and 2 testing, following test preparation and processing.

Test	Treatment (mg BaSO$_4$/kg reference soil d.w.)	Initial pH[1]	Final pH[2]	Initial Conductivity (µS/m)	Final Conductivity (µS/cm)	Initial Moisture Content (%)	Final Moisture Content (%)
Orchardgrass	Artificial Soil	7.52	7.71	220	385	34.0	[3]
	Reference Soil	7.66	8.04	195	407	15.3	[3]
	1000	7.86	8.09	215	428	15.9	[3]
	10000	7.92	8.12	231	373	14.5	[3]
	30000	7.94	8.09	232	472	15.7	[3]
	100000	7.97	8.11	227	490	14.1	[3]
	300000	8.01	8.11	232	621	13.8	[3]
	1000000	8.32	8.58	233	434	8.4	[3]
Perennial Ryegrass	Artificial Soil	7.54	8.16	221	301	31.9	[3]
	Reference Soil	7.94	8.29	193	343	15.8	[3]
	1000	7.98	8.29	214	364	13.1	[3]
	10000	7.99	8.26	228	436	15.9	[3]
	30000	8	8.26	219	449	15.6	[3]
	100000	7.99	8.27	222	455	14.8	[3]
	300000	8.01	8.41	230	293	13.4	[3]
	1000000	8.3	8.65	220	443	8.2	[3]

[1] measured at the beginning of the test

[2] measured at the end of the test

[3] moisture content measurements are not usually taken when conducting plant tests since irrigation changes the moisture content of the soil

[4] deionized water was added after soil m c measurements were taken at the beginning of the test

Table A.4. *Eisenia andrei* adult survival following 7 and 14 days of exposure to artificial soil, reference control site soil, and BaSO$_4$-amended reference site soil.

Treatment	Day 7		Day 14	
(mg BaSO$_4$/kg reference soil d w.)	Survival (n = 15)	Standard Error	Survival (n = 15)	Standard Error
Phase 1 Testing				
Artificial Soil	15	0	15	0
Reference Soil	15	0	15	0
500	15	0	15	0
1500	15	0	15	0
5000	15	0	15	0
10,000	15	0	15	0
25,000	15	0	15	0
50,000	15	0	15	0
Phase 2 Testing				
Artificial Soil	15	0	15	0
Reference Soil	15	0	15	0
1000	15	0	15	0
10000	15	0	15	0
30000	15	0	15	0
100000	15	0	15	0
300000	15	0	15	0
1000000	15	0	15	0

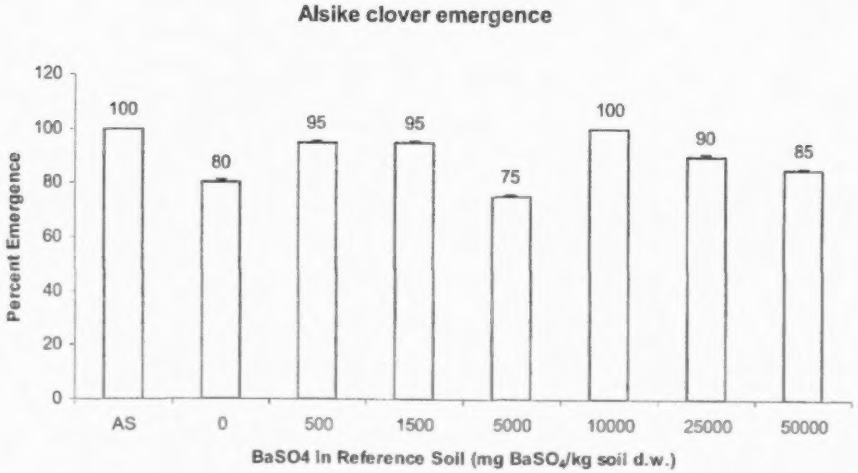

Figure A.1. Emergence of alsike clover following 14 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars indicate one standard error, and numbers above the columns indicate the percent emergence for each treatment.

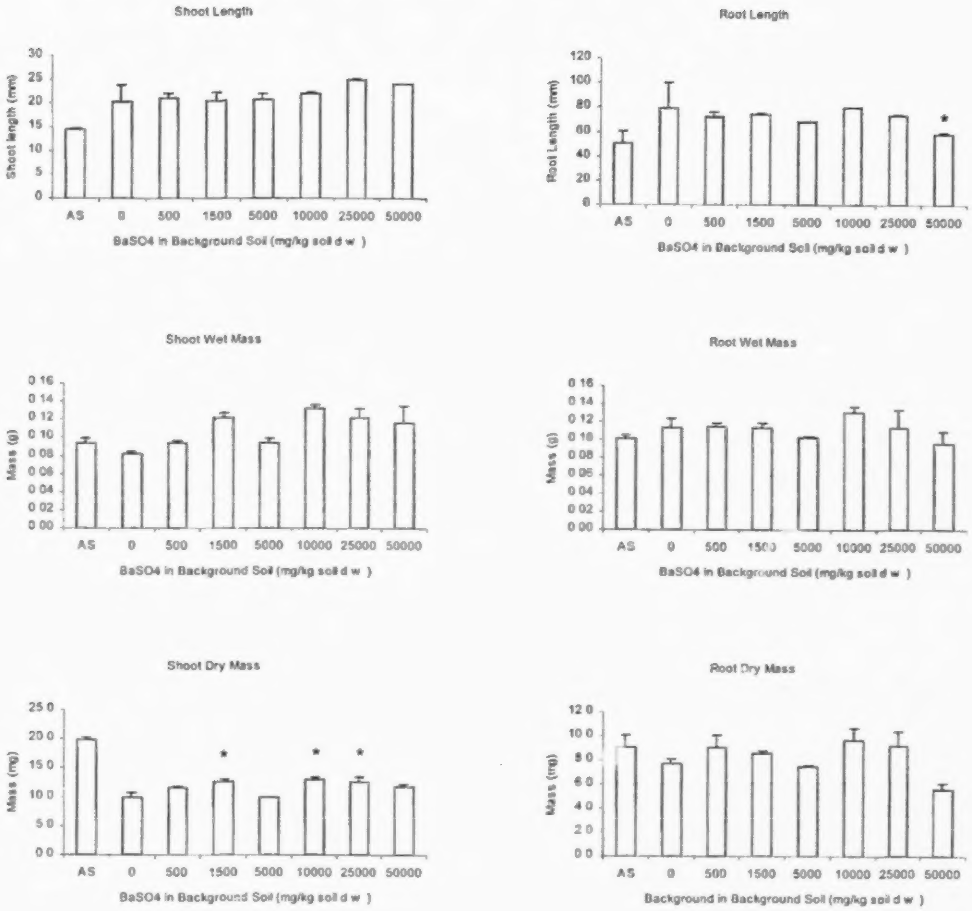

Figure A.2. Alsike clover seedling growth following 14 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars on the columns indicate one standard error, and asterisks indicate a significant difference from the reference control site soil.

Table A.5. The effect of exposure of alsike clover to barium sulphate-amended reference site soil, reference control soil, and artificial soil (Phase 1 test). Values in brackets indicate one standard error. Asterisks indicate values significantly different from those of the reference control soil treatment.

Treatment (mg BaSO4/kg reference soil d.w.)	**Endpoint**					
	Shoot Length (mm)	**Shoot Wet Mass** (g)	**Shoot Dry Mass** (mg)	**Root Length** (mm)	**Root Wet Mass** (g)	**Root Dry Mass** (mg)
Artificial Soil	14.60 (0.20)	0.09 (0.01)	19.90 (0.40)	50.70 (9.40)	0.10 (0.004)	9.10 (1.00)
Reference Soil	20.33 (3.44)	0.08 (0.03)	9.95 (0.75)	79.21 (21.06)	0.11 (0.01)	7.75 (0.35)
500	21.18 (0.96)	0.09 (0.003)	11.65 (0.15)	72.17 (4.21)	0.11 (0.004)	9.10 (1.00)
1500	20.70 (1.59)	0.12 (0.01)	12.50* (0.60)	73.91 (1.33)	0.11 (0.01)	8.65 (0.15)
5000	20.80 (1.38)	0.09 (0.01)	9.90 (0)	68.59 (0.17)	0.10 (0.0003)	7.45 (0.15)
10,000	22.15 (0.30)	0.13 (0.004)	13.05* (0.35)	79.95 (0.30)	0.13 (0.01)	9.75 (0.95)
25,000	24.84 (0.30)	0.12 (0.01)	12.70* (0.70)	73.24 (1.31)	0.11 (0.02)	9.20 (1.20)
50,000	24.27 (0.07)	0.12 (0.02)	11.85 (0.25)	58.05* (1.40)	0.10 (0.01)	5.60 (0.50)

Table A.6. Chemical analyses of the barium sulphate-amended reference soil.

Treatment (mg $BaSO_4$/kg soil d.w.)	**Total Barium*** (ppm)		**Barium as Saturated Paste** (mg/L)	
	Day 0	**Day 14**	**Day 0**	**Day 14**
Eisenia andrei				
Reference Soil	273	289	0.07	0.056
1000	720	714	<0.05	0.041
10000	1920	1570	<0.05	Not available
30000	1990	1630	<0.05	0.061
100000	2080	1620	<0.05	0.069
300000	1950	1330	<0.05	0.231
1000000	1470	1060	0.11	1.92
Perennial Ryegrass				
Reference Soil	284	1160	<0.05	0.172
1000	766	791	<0.05	0.073
10000	1830	1440	<0.05	0.071
30000	1910	1520	<0.05	0.055
100000	1830	1410	<0.05	0.067
300000	1570	1150	<0.05	0.134
1000000	1230	1060	0.30	2.40

*Samples analysed by ICAP, which is an acid digestion Barium sulphate is not soluble in acid, therefore the analytical values underestimate real soil $BaSO_4$ concentrations.

Table A.7. *Onychiurus folsomi* survival following 7 days of exposure to artificial soil, reference control site soil, and $BaSO_4$-amended reference site soil.

Treatment (mg $BaSO_4$/kg reference soil d.w.)	Survival (n = 30)	Standard Error
Artificial Soil	30	0
Reference Soil	30	0
1000	30	0
10000	30	0
30000	30	0
100000	30	0
300000	20*	0
1000000	30	0

* N = 20 for this treatment (one replicate test unit was destroyed)

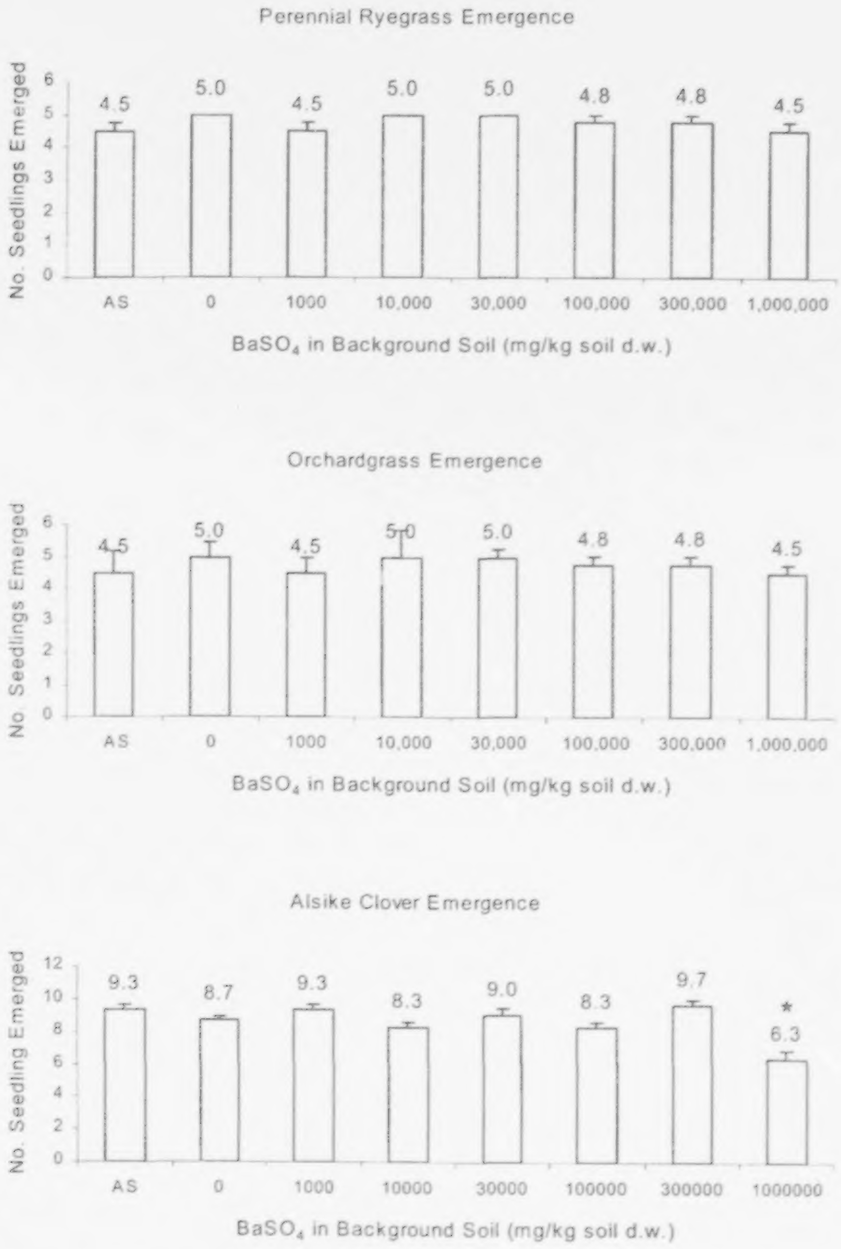

Figure A.3. Emergence of orchardgrass and perennial ryegrass following 14 days, and alsike clover following 21 days of exposure to the experimental control soil (artificial soil, AS), and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars indicate one standard error, and numbers above the columns indicate the number of emerged seedlings per treatment. Asterisks indicate significant differences from the reference control soil treatment.

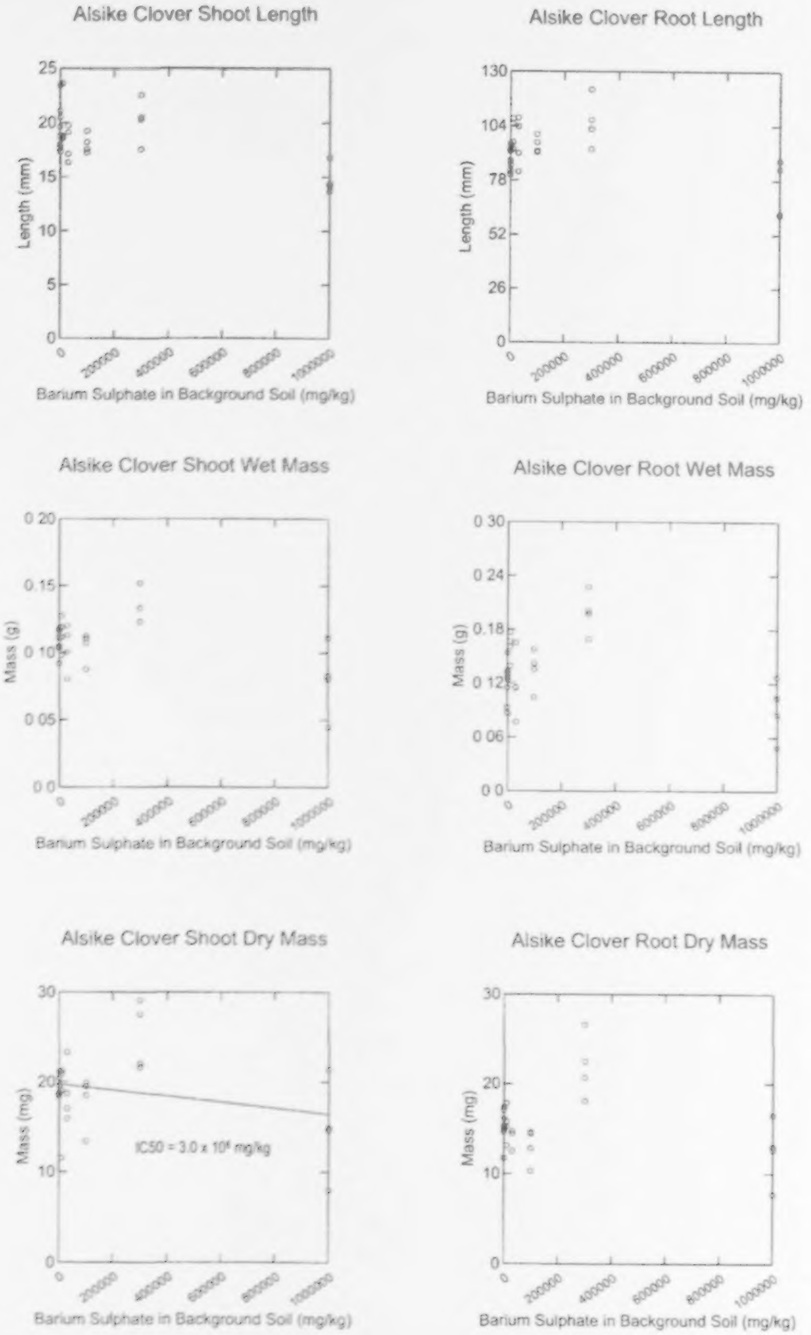

Figure A.4 . Alsike clover growth following 21 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

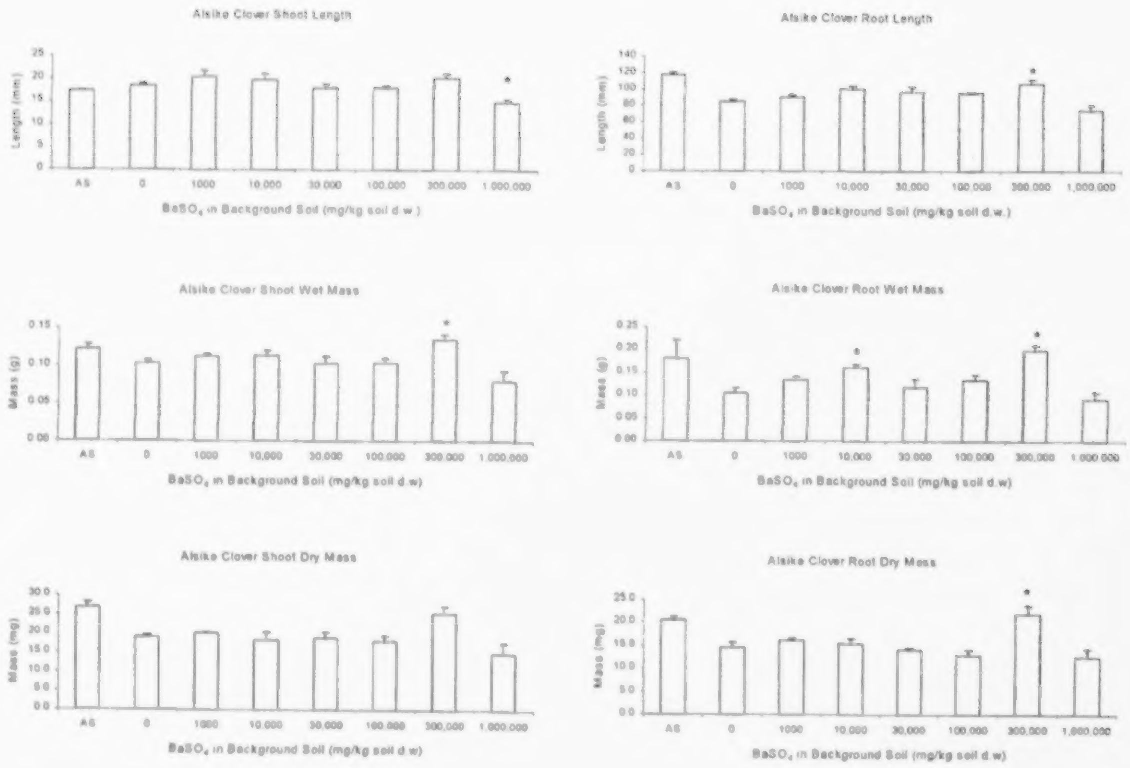

Figure A.5. Alsike clover growth following 21 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars above columns indicate one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

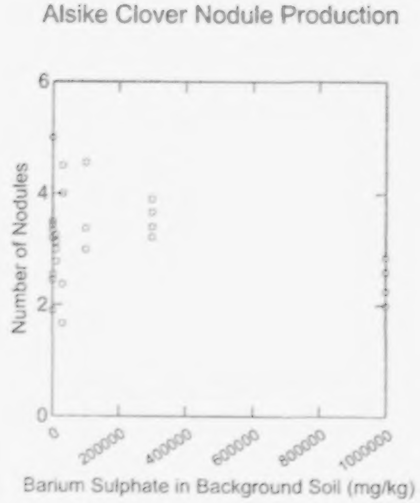

Figure A.6. Nodule production on the roots of alsike clover seedlings following 21 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

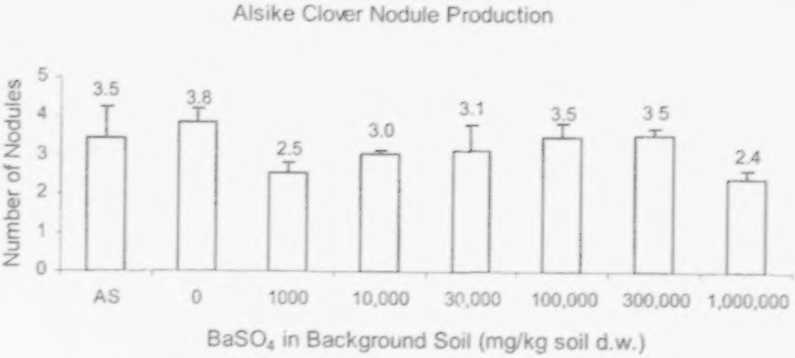

Figure days of exposure to the experimental control soil (artificial soil, AS), reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars above columns indicate one standard error and numbers above the columns indicate the number of nodules observed per treatment.

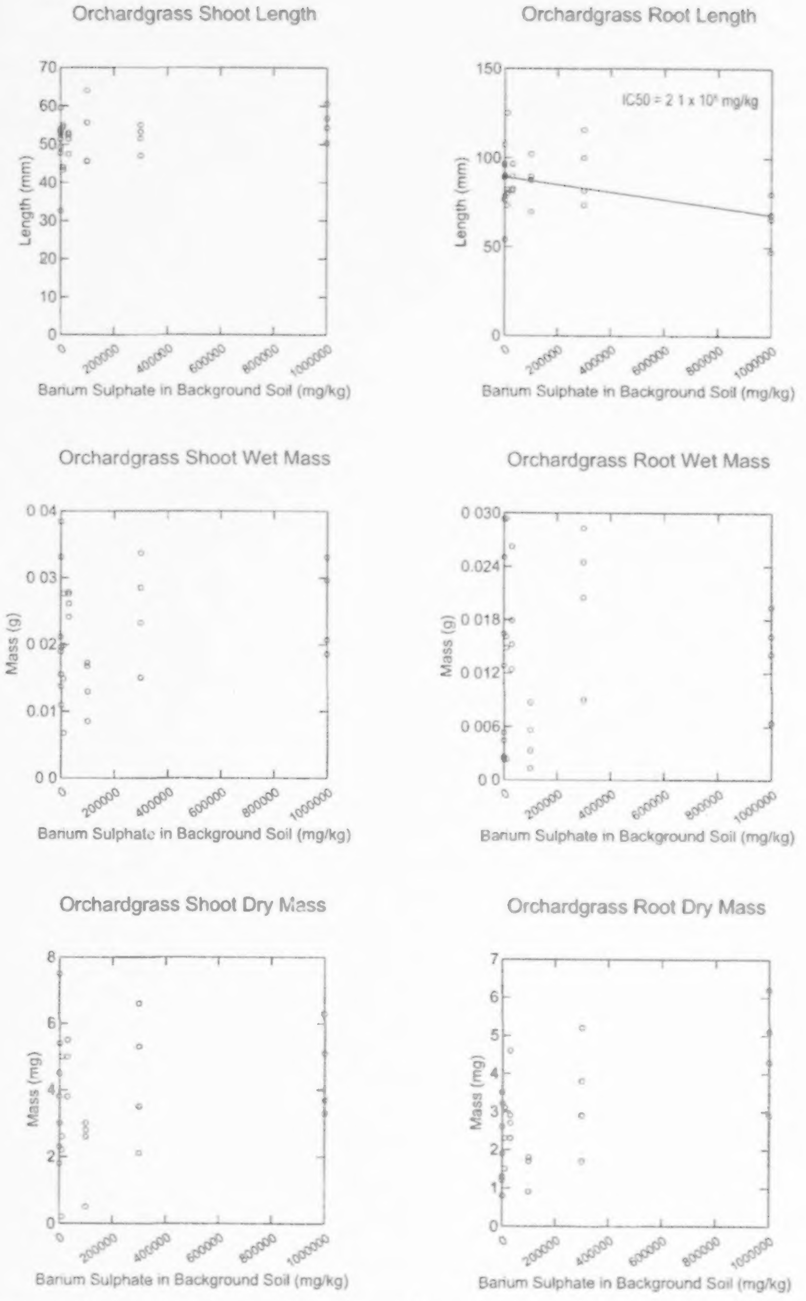

Figure A.8 . Orchardgrass growth following 14 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

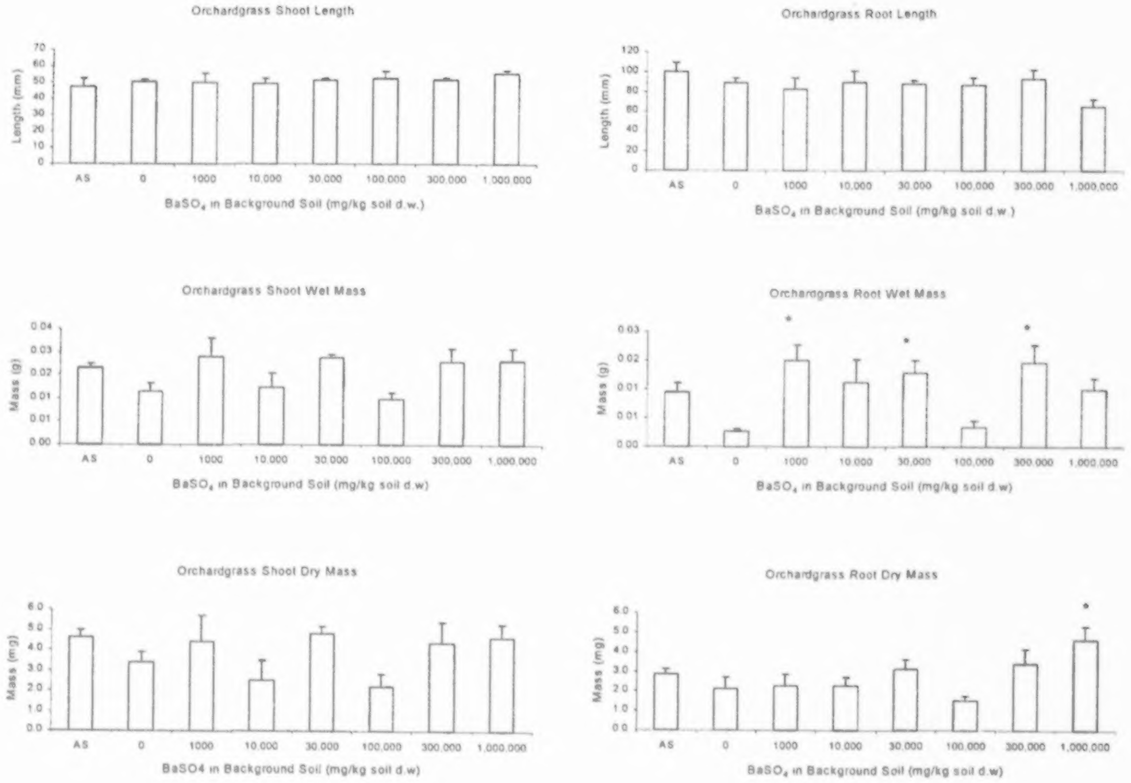

Figure A.9. Orchardgrass growth following 14 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars above columns indicate one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

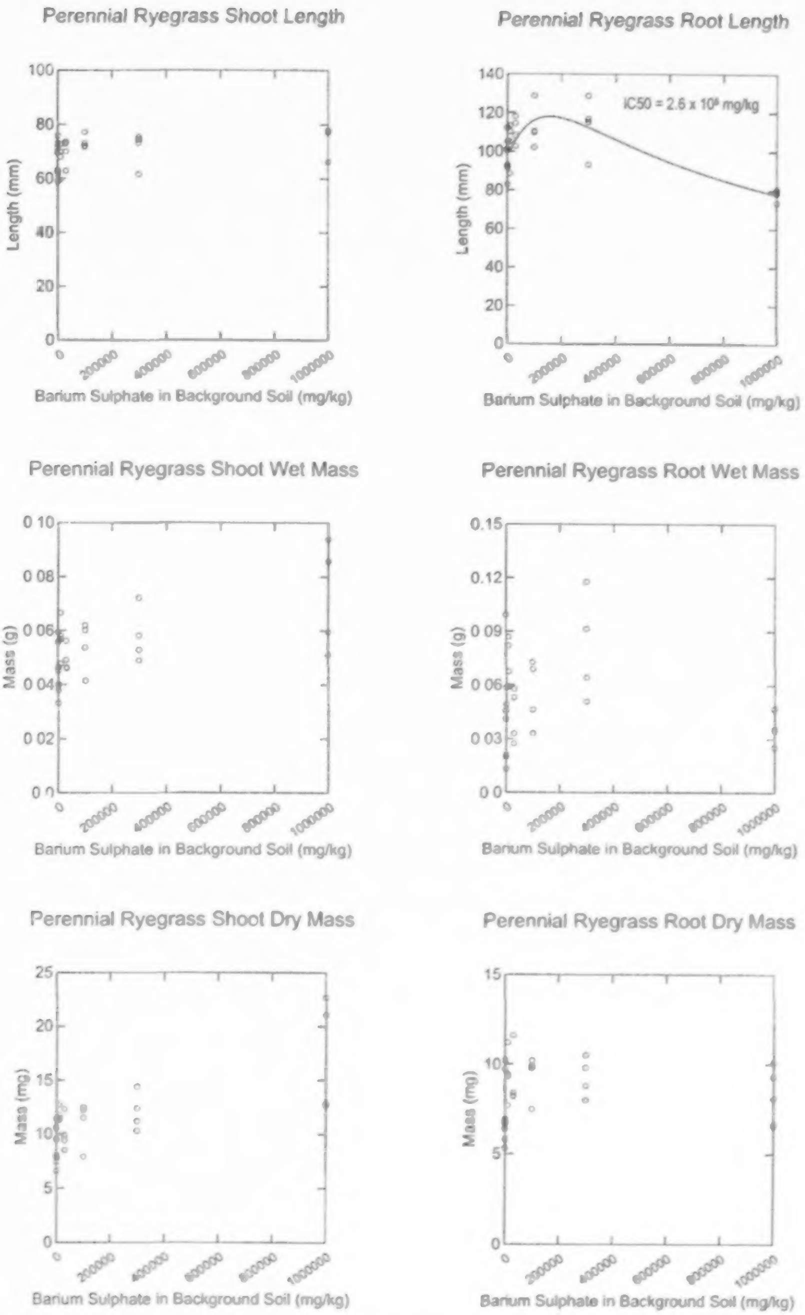

Figure A.10. Perennial ryegrass growth following 14 days of exposure to the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.).

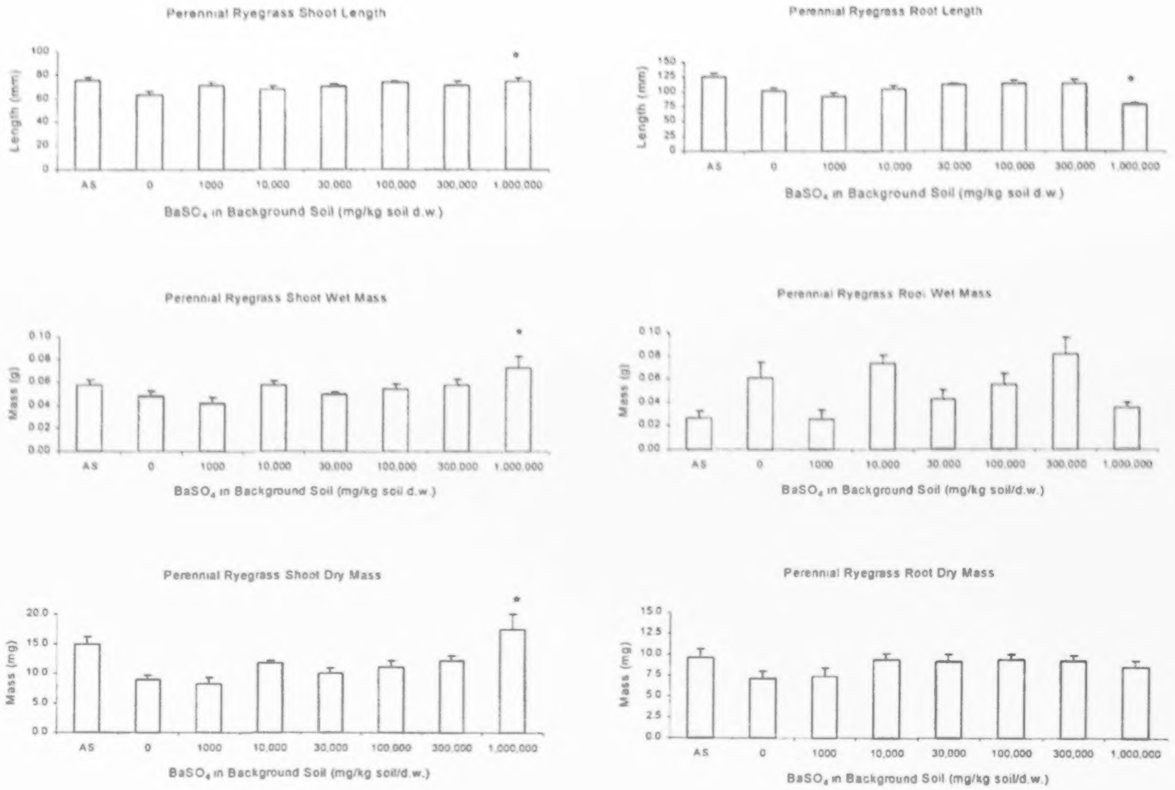

Figure A.11. Perennial ryegrass growth following 14 days of exposure to the experimental control soil (artificial soil, AS), the reference control site soil, and the barium sulphate-amended reference site soils (mg $BaSO_4$/kg soil d.w.). Bars represent one standard error. Asterisks indicate significant differences from the unamended reference control site soil.

Table A.8. Effect of exposure to barium sulphate-amended soil to the growth of alsike clover, orchardgrass and perennial ryegrass. Values in brackets indicate upper and lower confidence limits, respectively.

Species	Parameter	Model	IC50[1] (mg/kg)	IC20[1] (mg/kg)
Alsike clover	Shoot Dry Mass	Linear	> 1,000,000 (-1.2×10^6, 7.1×10^6)	> 1,000,000 (-4.6×10^5, 2.8×10^6)
Orchardgrass	Root Length	Linear	> 1,000,000 (4.0×10^5, 3.9×10^6)	850,000 (1.6×10^5, 1.5×10^6)
Perennial Ryegrass	Root Length	Hormesis	> 1,000,000 (5.7×10^5, 4.6×10^6)	960,000 (6.4×10^5, 1.3×10^6)

[1] Where confidence limits are negative, this indicates that the fit of the model to the data was poor

Table A.9. The effect of exposure of alsike clover, orchardgrass and perennial ryegrass to barium sulphate-amended reference site soil, reference control soil, and artificial soil (Phase 2 testing). Values in brackets indicate one standard error.

Treatment ($mg\ BaSO_4/kg$ reference soil d.w.)	Endpoint					
	Shoot Length (mm)	**Shoot Wet Mass** (g)	**Shoot Dry Mass** (mg)	**Root Length** (mm)	**Root Wet Mass** (g)	**Root Dry Mass** (mg)
Alsike Clover						
Artificial Soil	17.29 (0.35)	0.12 (0.01)	26.78 (1.29)	117.33 (2.66)	0.18 (0.04)	20.45 (0.84)
Reference Soil	18.52 (0.44)	0.10 (0.004)	19.00 (0.47)	85.54 (2.52)	0.11 (0.01)	14.55 (1.09)
1000	20.59 (1.26)	0.11 (0.003)	19.98 (0.50)	90.05 (2.34)	0.13 (0.01)	15.98 (0.52)
10000	19.90 (1.24)	0.11 (0.01)	18.15 (2.27)	100.33 (3.47)	0.16 (0.01)	15.48 (0.97)
30000	18.07 (0.81)	0.10 (0.01)	18.73 (1.63)	96.08 (5.91)	0.12 (0.02)	14.00 (0.05)
100000	18.07 (0.44)	0.10 (0.01)	17.83 (1.50)	94.78 (1.99)	0.13 (0.01)	13.03 (0.99)
300000	20.23 (1.03)	0.14 (0.01)	25.05 (1.91)	105.91 (5.94)	0.20 (0.01)	21.98 (1.79)
1000000	14.76 (0.70)	0.08 (0.01)	14.73 (2.74)	73.64 (6.92)	0.09 (0.02)	12.45 (1.81)
Orchardgrass						
Artificial Soil	47.78 (4.84)	0.023 (0.001)	4.60 (0.38)	100.69 (8.99)	0.013 (0.002)	2.87 (0.26)
Reference Soil	50.36 (1.21)	0.016 (0.002)	3.40 (0.48)	89.41 (4.68)	0.004 (0.001)	2.15 (0.55)
1000	49.70 (5.92)	0.027 (0.005)	4.43 (1.27)	82.43 (11.19)	0.021 (0.004)	2.28 (0.58)
10000	49.16 (3.20)	0.017 (0.004)	2.50 (0.98)	90.06 (11.80)	0.016 (0.006)	2.30 (0.40)
30000	51.08 (1.27)	0.026 (0.001)	4.83 (0.36)	87.58 (3.47)	0.018 (0.003)	3.13 (0.51)
100000	52.71 (4.45)	0.014 (0.002)	2.23 (0.58)	87.17 (6.66)	0.005 (0.002)	1.53 (0.21)
300000	51.69 (1.72)	0.025 (0.004)	4.37 (0.99)	92.71 (9.44)	0.021 (0.004)	3.40 (0.74)
1000000	55.52 (2.17)	0.026 (0.003)	4.60 (0.69)	65.38 (6.72)	0.014 (0.003)	4.63 (0.69)
Perennial Ryegrass						
Artificial Soil	75.05 (2.97)	0.058 (0.004)	15.00 (1.13)	125.45 (5.57)	0.03 (0.01)	9.65 (0.95)
Reference Soil	63.25 (2.21)	0.048 (0.004)	9.00 (0.65)	101.50 (4.14)	0.06 (0.01)	7.07 (0.94)
1000	70.74 (2.79)	0.042 (0.005)	8.33 (1.06)	93.20 (4.63)	0.03 (0.01)	7.37 (0.97)
10000	67.45 (2.85)	0.057 (0.004)	11.78 (0.32)	104.20 (5.53)	0.07 (0.01)	9.40 (0.72)
30000	69.80 (2.46)	0.049 (0.002)	10.03 (0.81)	110.80 (3.40)	0.04 (0.01)	9.10 (0.83)
100000	73.49 (1.27)	0.054 (0.005)	11.03 (1.06)	112.83 (5.65)	0.06 (0.01)	9.35 (0.62)
300000	71.16 (3.22)	0.058 (0.005)	12.08 (0.89)	113.49 (7.41)	0.08 (0.01)	9.27 (0.55)
1000000	74.63 (2.79)	0.073 (0.010)	17.33 (2.66)	78.03 (1.51)	0.04 (0.004)	8.52 (0.76)

APPENDIX IV

STANTEC (2003)

BARITE SOIL GUIDELINES PROJECT

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Prepared for:

Barite Soil Guidelines Technical Steering Committee

September 2003

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APPENDICES

APPENDIX A RESULTS OF THE SITE SOIL ASSESSMENT: ECOTOXICITY EVALUATION WITH PLANT AND INVERTEBRATE SPECIES

ECOTOXICITY EVALUATION OF REFERENCE SITE SOILS AMENDED WITH BARIUM ACETATE

1 Introduction

In March 2003, ESG International (now Stantec Consulting Ltd.) was contracted by Chevron Canada Resource to provide an ecotoxicity assessment of site soils contaminated with a mixture of petroleum hydrocarbons and barium sulphate. The soils originated from the drilled and abandoned Chevron Canada Resources CDN SUP Waterton 04-13-05-01 W5M wellsite, located near Pincher Creek, Alberta. The results of the acute earthworm test indicated that the contaminated site soil was not acutely toxic to earthworms; however, there was significant reduction in growth of two of the three plant species tested, following longer-term exposure to the contaminated soil. Petroleum hydrocarbon soil concentrations in the contaminated soil tested were not at levels expected to have caused the adverse effects. The barium sulphate soil concentration, as total barium sulphate, in the contaminated soil was at levels sufficiently high to potentially cause adverse effects; however, the level of barium sulphate as saturated paste, which represents the water soluble fraction, was extremely low. As a result, Chevron Canada, on behalf of the Canadian Association of Petroleum Producers (CAPP), contracted ESG to provide a preliminary ecotoxicity assessment of the previously-collected reference control site soil amended with barium acetate. Barium acetate was chosen as the toxicant because it is more soluble than barium sulphate and more representative of a worse-case exposure scenario. The reference control site soil is a fine-grained Alberta till (very clayey) soil that was collected by collected by Komex International Inc. from a site in Alberta. The physical and chemical characteristics of the reference control soil were similar to that of the contaminated site soil, but the soil was free of contamination.

The preliminary toxicity assessment included three experiments: an acute earthworm toxicity test, an acute collembola toxicity test and an acute plant screening test. The experiments were conducted with reference control site soil (e.g., background soil) amended with different quantities of industrial-grade barium acetate, as well as with an experimental control soil (e.g., artificial soil). The worm and plant test species were identical to those used in the previous testing program with the contaminated site soil; acute tests were also conducted with a soil arthropod (a collembola, or springtail, species). The test species included the earthworm *Eisenia andrei* (compost worm), the collembola *Onychiurus folsomi* and the leguminous plant, alsike clover (*Trifolium hybridum*). These species had been originally chosen because *E. andrei* and collembola are commonly used to assess the effects of contaminated soil on terrestrial invertebrates, and alsike clover is used as a forage crop on rangeland in Alberta. Following the preliminary toxicity assessment, chronic tests were performed to assess the effects of longer exposure durations. In addition to those test species listed above, tests with perennial ryegrass (*Lolium perenne*) and orchard grass (*Dactylis glomerata*) were also conducted.

The reference control soils (Background-2) were collected by Komex International and received by ESG on February 18 and March 14, 2003. After their arrival at ESG's Southgate Laboratory, the soil samples (e.g., 20-L buckets) were assigned identification numbers and were entered into a logbook (Table A.1; Appendix A). Five buckets of the background soil were received, and the samples were denoted as Background-2 (1-5). Prior to testing, the soil samples were air-dried for 24 hours, broken up by hand, pulverized into smaller aggregates, and sieved through a 6-mm stainless steel sieve. Once all the soil from the five buckets had been sieved, they were homogenized and stored in plastic 20-L buckets at room temperature (20 ± 2°C) until use. The soil moisture content of the background and artificial soils was determined prior to testing.

The experimental control soil was an artificial soil (AS) formulated in the laboratory, and characterized as a fine sandy loam. The physical and chemical characteristics of the experimental control soil are listed in Table A.2 (Appendix A).

The objective of this research was to determine the concentration of barium acetate in the reference control site soil (Background-2 soil) that would adversely affect earthworm and collembola survival and seedling emergence and early growth. The experimental control soil provided QA/QC data on test organism health and experimental conditions. Testing began on May 14, 2003. The results of the testing are summarized and presented in this report .

2 Chemical Analysis

Barium soil concentration of the $Ba(C_2H_3O_2)_2$-amended soils was measured at the beginning (Day –1 for earthworms, Day 0 for plants) and at the end (Day 14 for earthworms, Day 21 for plants) of plant and earthworm tests. Soil subsamples were collected from each amended treatment at the beginning and end of the *Eisenia andrei* acute tests and the beginning and end of the *Eisenia andrei* chronic and alsike clover definitive tests. Samples were collected from the two different species to observe the effect, if any, of the presence of earthworms *vs.* plants on the total or water-soluble concentration of barium in the soil. Barium acetate was analysed as total soil concentration and as saturated paste. Barium acetate is soluble and, as a result, the $Ba(C_2H_3O_2)_2$-saturated paste values were expected to represent the water-soluble fraction of barium in the soil. The chemical analyses were performed by Philip Analytical Services Corporation, Mississauga, Ontario.

The total barium and barium as saturated paste concentrations in the soil samples collected from the *Eisenia andrei* (acute and definitive) and alsike clover (definitive) tests are presented in Table A.3 (Appendix A). There was no discernable effect of test species (i.e., plant vs. earthworm) on any of the measured soil concentrations between the *E. andrei* and alsike clover end-of-test results.

For all three sets of analyses, the reference control soil (0 mg/kg) had relatively high background total barium concentrations that ranged from 239 to 268 mg/kg soil d.w.. The measured concentrations are in good agreement with the nominal concentrations once background barium concentrations are accounted for, since 255.43 g (molecular weight) barium acetate is equivalent to 137.33 g (molecular weight) of barium. Nominal barium soil concentrations were regressed against measured barium concentrations in soil analyzed from the beginning and end of the earthworm acute and plant definitive tests, and from the beginning of the earthworm chronic test. The measured values of total barium were corrected for background barium concentrations, and the results show an increasing concentration gradient that was strongly correlated with nominal barium exposure concentrations (Figures A.1 to A.3; Appendix A). Regression co-efficients (r^2 values) for all sampling times were equal to or greater than 0.999.

The concentration of barium measured as saturated paste did not correspond well to either the nominal barium soil concentrations or the measured total barium concentrations for all three sets of analyses. A concentration gradient existed among soil treatments sampled at the beginning (Day –1) of the acute earthworm test (Table A.3; Appendix A) but not in soils sampled at the end (Day 14) of the test. No obvious patterns or trends were discerned among treatments of the soils sampled at the beginning or end of the definitive plant test, nor in soil sampled at the beginning of the chronic earthworm test (Table A.3; Appendix A).

Samples of soil treatments were only analyzed from the beginning of the earthworm chronic test. No samples were collected on the last day of the test. Based on the results shown in Table A.3 (Appendix A), it was decided that the saturated paste extraction of barium was not a good surrogate for the water-soluble, or bioavailable, fraction of barium in soil.

3 Acute Toxicity To *Eisenia andrei*

3.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 14-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-h day, 8-h night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 2000, 4000, 6000, 8000, 10,000, and 12,000 mg $Ba(C_2H_3O_2)_2$/kg soil d.w.

Test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 g soil wet weight (w.w.) of test soil, for both the AS and Background-2 reference soils. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 3 replicate test units. Five adult earthworms were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998a).

3.2 SOIL PREPARATION

On Day -1, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting $Ba(C_2H_3O_2)_2$-soil mixture (referred to as BaAc) was then hydrated with reverse osmosis (R.O.) water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 17 to 35%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

On Day 0, five clitellate *Eisenia andrei* were added to each test unit and the soils in the test units were misted gently with reverse osmosis (R.O.) water as necessary.

Substantially more water was added to the test units of the reference soil treatments after the earthworms were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of R.O. water while homogenizing the soil; therefore, most of the water was added immediately after the earthworms were added.

3.3 STATISTICAL ANALYSES

Acute earthworm test results were analyzed using the Trimmed Spearman-Karber regression analysis (Stephan, 1989) to generate 7- and 14-d LC50 estimates and their 95% confidence limits.

3.4 RESULTS

On Day 7, each test unit was inspected to determine the number and condition of surviving *E. andrei.* The 7-d LC50 was 7673.4 mg BaAc/kg soil d.w. and the associated 95% confidence limits were 7193.3 and 8185.6 mg/kg soil d.w.. The 14-d LC50 was 7168.2 mg BaAc/kg soil d.w. with 95% confidence limits of 6853.9 and 7496.9 mg/kg soil d.w. (Table A.5; Appendix A). All earthworms in each treatment up to and including 6000 mg/kg BaAc soil d.w. were alive. In the 8000 mg/kg BaAc soil d.w. treatment, only 6 worms remained alive (n=15); and all surviving worms appeared unhealthy. All the worms in 10,000 and 12,000 mg/kg treatments were dead after 7 days. (Table A.5; Appendix A).

On Day 14, each test unit was inspected again to determine the number and condition of surviving *E. andrei.* All earthworms in each treatment up to and including 6000 mg/kg BaAc soil d.w. were still alive; however, most worms had re-sorbed their clitellums, a sign of stress. In the 8000 mg/kg BaAc soil d.w. treatment, only 2 worms remained alive, and appeared to be unhealthy with re-sorbtion of their clitellum, pinching-off in the midsection of their body and lethargic movement (Table A.5; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg/kg BaAc soil d.w.), and BaAc-amended reference soils. The addition of barium acetate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *E. andrei* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil increased because of the addition of barium acetate, and reached 2050 µS/cm at the highest concentrations, (Table A.4; Appendix A) which is outside of the range considered acceptable for *E. andrei* survival and growth (earthworm growth has been observed to be unaffected up to 1500 µS/cm).

4 Acute Toxicity To *Onychiurus folsomi*

4.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- 7-day duration;
- Test endpoint: adult survivorship
- Temperature: constant at 20 ± 2°C; and,
- Photoperiod: 16-h day, 8-h night (fluorescent illumination).

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 2000, 4000, 6000, 8000, 10,000, and 12,000 mg $Ba(C_2H_3O_2)_2$/kg soil d.w.

Test units consisted of 125-mL, wide-mouthed glass mason jars filled with 30 g soil wet weight (w.w.) of test soil, for both the AS and Background-2 reference soils. Jars were covered with metal lids screwed on very lightly by a metal screw ring to facilitate gas exchange. Each treatment included 3 replicate test units. Ten (10) adult collembola were added to each test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998c).

4.2 SOIL PREPARATION

On Day -1, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the soils ranged from 17 to 35%. Physical and chemical soil parameters such as pH, electrical conductivity, and moisture content were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

On Day 0, 10 adult *Onychiurus folsomi* were added to each test unit and the soils in the test units were misted gently with R.O. water as necessary. Substantially more water was added to the test units of the reference soil treatments after the collembola were added to the test units, as compared to the experimental control soil treatment. The extremely clayey nature of the site soils precluded the addition of the required volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the collembola were added.

4.3 STATISTICAL ANALYSES

No statistical analyses of the collembola toxicity data were conducted. The data sets and their individual histograms provided a visual estimate of the LC50.

4.4 RESULTS

On Day 7, each test unit was inspected to determine the number and condition of surviving *O. folsomi.* No apparent difference in mortality in any of the treatments following 7 days of exposure, and all surviving collembola appeared healthy (Table A.6; Appendix A). Soil pH, electrical conductivity and soil moisture content were measured and compared among the experimental control, reference control (0 mg/kg BaAc soil d.w.), and BaAc-amended reference soils. The addition of barium acetate to the reference soil did not affect soil pH, and all soil pH values were within a range acceptable for *O. folsomi* survival and growth. Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments. Soil electrical conductivity in the reference soil was increased by the addition of barium acetate, reaching a value of 2050 µS/cm (Table A.4; Appendix A).

5 Preliminary Screening Plant test

5.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organism was alsike clover from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d;
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 300-350 ΦE/m^2/sec;

Test endpoint: seedling emergence, shoot and root wet and dry mass and number of nodules.

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series; 500, 1000, 2000, 4000, 6000, 8000, 10,000 and 12,000 mg BaAc/kg soil d.w.

Test units consisted of a glass petri dish enclosed within an inverted 1-L clear polypropylene container. Each petri dish was filled with 100 g w.w. of AS at 35% moisture content or 100 g w.w. of reference ("Background-2") soil at 17% moisture content. Both AS and reference soil treatments consisted of 2 replicate test units. Ten alsike clover seeds were planted in each test unit. Methods and procedures for site soil amendment are described in detail in EC, 1998b.

5.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis, but volumes were adjusted to compensate for differences in bulk density of the soils. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with R.O. water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked daily and watered on an as-need basis with well-aerated de-chlorinated, municipal tap water.

5.3 TEST PROCESSING

On Day 14, each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than 3 mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment, was recorded.

5.4 STATISTICAL ANALYSES

No statistical analyses of the alsike clover toxicity data were conducted. The data histograms provided a visual estimate of the EC50s.

5.5 RESULTS

Alsike clover emergence in the reference soil was adversely affected at the 2000 mg BaAc/kg soil d.w. treatment and greater (Figure A.4; Appendix A).

In general, seedling growth was adversely affected at the 1000 mg BaAc/kg soil d.w. treatment and greater compared to the reference control soil (0 mg BaAc /kg soil d.w.) (Table A.7, Figure A.5; Appendix A). Adverse effects on shoot length began at 500 mg/kg soil d.w., while shoot dry mass and number of nodules were affected at 2000 mg/kg soil d.w. and greater.

Soil pH and electrical conductivity were measured and compared among the test soils, and the values for both parameters in all soil treatments were within the range that supports plant growth. The addition of barium acetate to the reference soil did not affect pH or conductivity (Table A.4; Appendix A).

6 Definitive Plant Toxicity Tests

6.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static acute;
- Test organisms were alsike clover, perennial ryegrass and orchard grass from Pickseed Canada Inc., Sherwood Park, AB;
- Test duration: 14 d for perennial ryegrass and orchard grass; 21 d for alsike clover
- Temperature: 24/16 ± 2°C day/night;
- Photoperiod: 16/8-h day/night ;
- Light intensity: ~ 300-350 $\Phi E/m^2/sec$;

Test endpoint: seedling emergence, shoot and root wet and dry mass (phytotoxicity and number of nodules for alsike clover)

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series: 75, 150, 300, 500, 1000, and 2000 mg BaAc/kg soil d.w.

Test units consisted of a 1-L clear polypropylene container filled with 485 g w.w. of AS at 35% moisture content or 375 g w.w. of reference ("Background-2") soil at 17% moisture content. Both the AS and reference soil control treatments consisted of 6 replicate test units. The 75, 150, and 300 mg BaAc/kg soil d.w. treatments each had 4 replicate test units, whereas the 500 1000 and 2000 mg BaAc/kg soil d.w. treatments each had 3 replicate test units.

Ten alsike clover seeds were planted in each test unit, while orchard grass and perennial ryegrass both had five seeds per test unit. Methods and procedures for site soil amendment are described in detail elsewhere (EC, 1998b).

6.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the seeds were planted. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A). Seeds were planted in each test unit, to a depth of approximately the length of the longest side of the seed. Test units were then misted gently with R.O. water. Substantially more water was added to the test units containing the clayey background soil as compared to the experimental control soil. Test units were checked daily and watered on an as-need basis with well-aerated de-chlorinated, municipal tap water.

6.3 TEST PROCESSING

On the final testing day (Day 14 for orchard grass and perennial ryegrass; Day 21 for alsike clover) each test unit was inspected to determine the number and condition of emerged seedlings. A seedling was considered emerged if the shoot tip was greater than 3 mm above the surface of the soil. Seedlings were carefully removed from the soil and any soil particles adhering to the roots were gently washed from each seedling using a spray bottle. Once cleaned, seedling shoot and root length were measured as well as shoot and root wet mass. For alsike clover, two more endpoints were measured: phytotoxicity and number of nodules. The roots of alsike clover plants were inspected after length measurements were taken, and any obvious effect on the number of nodules present per plant, per treatment was recorded. Plant material was dried for 48 hours at 90°C after which shoot and root dry mass was also determined.

6.4 STATISTICAL ANALYSES

Analyses of variance (ANOVA) procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended Background-2 soil) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil to determine the no-observable-adverse-effect concentrations (NOAEC) and lowest-observable-adverse-effect concentrations (LOAEC) (SPSS, 1997). Fisher's Least Significant Difference Test, using pairwise comparison probabilities, was used to determine significant differences among treatment means (SPSS, 1997). Linear or non-linear regression procedures were also applied to the data in order to generate IC50 and IC20 estimates. The regression analyses consisted of using a linear or four nonlinear regression models (i.e., logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variances among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998b) or Stephenson *et al.* (2000).

6.5 RESULTS

6.5.1 Alsike Clover

Emergence in the control soils was 85%. Emergence in the amended soils was adversely affected relative to that in the control soil at the treatment concentration of 1000 mg BaAc/kg soil d.w.) (Figure A.6; Appendix A). In general, number of nodules, and shoot- and root -length were the least sensitive endpoints for alsike clover. Root wet- and dry mass were the most sensitive endpoints (Figures A.7 to A.9; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and LOAEC estimates for alsike clover are summarized in Tables A.8a and b (Appendix A).

6.5.2 Orchard grass

Emergence in the control soil was 70%. Emergence in the amended soils was adversely affected relative to that in the control soil at the treatment concentration of 1000 mg BaAc/kg soil d.w.) (Figure A.6; Appendix A). The least sensitive endpoints for orchard grass were shoot and root length, followed by shoot dry mass and root wet mass. Shoot wet mass and root dry mass were the most sensitive endpoints (Figures A.10 and A.11; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and LOAEC estimates for orchard grass are summarized in Tables A.9a and b (Appendix A).

6.5.3 Perennial ryegrass

Emergence in the control soils was 87%. Emergence in the amended soils was adversely affected, but not significantly, relative to that in the control soil at the treatment concentration of 2000 mg BaAc/kg soil d.w. (Figure A.6; Appendix A). In general, shoot and root length were the least sensitive endpoints. Shoot wet and dry mass were the most sensitive endpoints (Figures A.12 and A.13; Appendix A). The ICp values (IC20, IC50) and their associated 95% confidence limits, and NOAEC and

LOAEC estimates for orchard grass are summarized in Tables A.10a and b (Appendix A).

For all three plant tests, soil pH and electrical conductivity were measured and compared among the test soils. The addition of BaAc to the reference soil affected both pH and conductivity; however, changes in both these parameters were well within the range that supports healthy plant growth (Table A.4; Appendix A).

7 Definitive Invertebrate Toxicity Tests

7.1 EXPERIMENTAL DESIGN AND CONDITIONS

The experimental design and conditions of the test were as follows:

- Test type was static chronic;
- Test organisms were *Eisenia andrei* and *Onychiurus folsomi* from in-house cultures;
- Test duration: 35 d for *O. folsomi*; 63 d for *E. andrei*;
- Temperature constant at 20 ± 2°C day/night;
- Photoperiod: 16/8-h day/night (fluorescent illumination); and,
- Test endpoints: adult survivorship, adult fecundity, number of juveniles

Experimental treatments included an experimental control soil (AS), an unamended reference ("Background-2") soil, and batches of reference ("Background-2") soil amended with sufficient barium acetate to achieve the following concentration series for *E. andrei*: 10, 30, 60, 100, 300, 600, 1000, 3000, 6000 mg BaAc/kg soil; and 100, 300, 600, 1000, 3000, 6000, 8000, 10 000, and 12,000 mg BaAc/kg soil d.w. for *O. folsomi.*

E. andrei test units consisted of 500-mL, wide-mouthed glass mason jars filled with 270 g soil wet weight (w.w.) of all test soils. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Each treatment included 10 replicate test units. Two adult worms were added to each test unit.

O. folsomi test units consisted of 125-mL, wide-mouthed glass mason jars filled with 30 g soil wet weight (w.w.) for all test soils. Jars were covered with metal lids screwed on very lightly by a metal screw ring to facilitate gas exchange. Each treatment included 10 replicate test units. Ten (10) adult collembola were added to each test unit.

Methods and procedures for site soil amendment are elsewhere (EC, 1998a and c).

7.2 SOIL PREPARATION

On Day 0, the control and amended soils were prepared on a dry weight basis. Barium acetate was added as a solid to dry reference soil and the soil was thoroughly homogenized using a mechanical mixer. The resulting BaAc-soil mixture was then hydrated with R.O. water and further homogenized. Because of the different water-holding capacities among the test soils, the moisture content of the prepared test soils ranged from 17 to 35 %. In addition, the extremely clayey nature of the background soil precluded the addition of the optimal volume of R.O. water while homogenizing the soil, therefore most of the water was added immediately after the organisms were added by gentle misting with R.O. water. Substantially more water was added to the test units containing the clay background soil as compared to the experimental control soil. Test units were checked, watered with R.O. and fed with oatmeal on a weekly basis. Physical and chemical soil parameters such as pH and electrical conductivity were measured following test soil preparation and at the end of the test (Table A.4; Appendix A).

7.3 TEST PROCESSING

On the final testing day for *O. folsomi*, (Day 35) each test unit was inspected to determine the condition and surviving number of adult collembola, along with number of juveniles produced. An adult was considered alive if it showed signs of movement within a 1-2 minute period. If a juvenile collembolan showed no immediate sign of movement, it was not enumerated. The collembola were floated to the top of each test unit by pouring water into the test unit until the soil surface was covered, then carefully removed with a very fine paintbrush and placed into a petri dish to be enumerated.

E. andrei adults were removed on Day 35 and the number of surviving adults were recorded. On day 63 the test was processed to determine the number of juveniles produced. Juvenile wet and dry mass were also measured, after the juveniles were dried for 48 hours at 90°C

7.4 STATISTICAL ANALYSES

O. folsomi: Analyses of variance procedures were applied to the data and a two-tailed Dunnett's test was used to compare each treatment mean to the mean of the reference (unamended, Background-2) control soil treatment. The Dunnett's pairwise comparison test was used to compare treatment means of the amended reference soils to the control reference soil to determine the no-observable-adverse-effect concentrations (NOAEC) and lowest-observable-adverse-effect concentrations (LOAEC) (SPSS, 1997). Fisher's Least Significant Difference Test, using pairwise comparison probabilities, was used to determine where the significant differences among treatment means occurred (SPSS, 1997). Linear or non-linear regression procedures were also applied to the data in order to generate IC50 and IC20 estimates. The regression analyses consisted of using a linear or four nonlinear regression models (i.e., logistic, gompertz, exponential and logistic with hormesis) that had been re-parameterized to include the ICp and the associated 95% confidence limits. The ICp is the inhibition concentration (IC) resulting in a specified percentage (p) effect. The data were examined for normality and homogeneity of variances among treatments. All analyses were performed with SYSTAT 7.0.1 (SPSS, 1997). A more detailed description of the statistical procedures used to analyze the definitive plant toxicity test data can be found in either Environment Canada (1998) or Stephenson *et al.* (2000).

E. andrei: no statistical analysis was performed on the definitive *E. andrei* test due to deficiencies in the data set (see Subsection 7.5.2).

7.5 RESULTS

7.5.1 *O. folsomi*

O. folsomi adults survived in the highest treatment of 12,000 mg/kg BaAc soil d.w.; however, the number of surviving adults started to decline significantly in the 6000 mg/kg treatment and greater. Juvenile production was adversely affected at 1000 mg/kg BaAc soil d.w., and there were no juveniles at the highest treatment level of 12, 000 mg/kg BaAc soil d.w. Fecundity was adversely affected at 1000 mg/kg BaAc soil d.w. and greater (Figures A.14 and A.15; Appendix A). Estimates of toxicity (EC20, EC50, NOAEC and LOAEC) are summarized in Tables A.11a and b (Appendix A).

7.5.2 *E. andrei*

Adults showed a slight decline in survival in the highest treatment of 6000 mg BaAc/kg soil d.w. Both juvenile production and adult fecundity decreased relative to the control (Figure A.16; Appendix A) at 1000 mg BaAc/kg soil d.w. and was absent at 3000 mg BaAc/kg soil d.w. In the experimental control soil (AS) the mean number of juveniles produced was 23.5. This meets the Environment Canada draft criteria for test acceptability (EC, 2002). In the unamended reference control soil ("Background–2") the mean number of juveniles produced was 1.2. Adult fecundity was also very low in the reference control soil. The low numbers of juveniles and fecundity in both the amended and control site soil indicates that this soil provides an unsuitable habitat for *E. andrei* reproduction.

For the two invertebrate tests, soil pH and electrical conductivity were measured and compared among the test soils. The addition of BaAc to the reference soil affected both pH and conductivity in both invertebrate tests; however, changes in both these parameters were well within values that support *O. folsomi* and *E. andrei* reproduction and survival (Table A.4; Appendix A). Soil moisture content (end of test) was comparable among all treatments, and was very similar among the reference soil treatments.

8 Discussion

The results of the acute invertebrate testing indicate that the reference site soil amended with barium acetate up to concentrations of 12,000 mg BaAc/kg soil d.w. was not acutely toxic to collembola, but 7- and 14-d LC50s were estimated for earthworms at 7673 and 7168 mg BaAc/kg soil d.w., respectively. The results of the acute screening plant test indicate that the reference site soil amended with barium acetate was acutely toxic to alsike clover with adverse effects observed at concentrations ≥ 1000 mg BaAc/kg soil d.w. and greater.

Generally, the results of the definitive plant testing show that adverse effects were observed at contaminations ≥ 500 mg BaAc/kg soil d.w, for all three species. Alsike clover appeared to be slightly more sensitive than perennial ryegrass or orchard grass but the difference was not great. IC50s for all plant species and endpoints ranged from 529 to 1892 mg BaAc/kg soil d.w.

O. folsomi adult fecundity and reproduction were adversely affected at concentrations between 600 and 1000 mg BaAc/kg soil d.w.. Adult survivorship was adversely affected at contaminations ≥ 6000 mg BaAc/kg soil d.w.

The results of the definitive earthworm testing indicate that the physical and/or chemical properties of the test soil were not conducive to *E. andrei* reproduction. At the treatment level of 1000 mg BaAc/kg soil d.w both adult fecundity and reproduction decreased, relative to the control.

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APPENDIX V

EXTRACTABLE BARIUM
SOIL CONTACT GUIDELINE

APPENDIX V – EXTRACTABLE BARIUM SOIL CONTACT GUIDELINE

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1. INTRODUCTION

The toxicity data in Stantec (2003) (Appendix IV) were presented in terms of the nominal concentration of barium acetate added. This Appendix describes how these toxicity data were converted from a nominal barium acetate basis to an extractable barium basis, and how the soil contact guidelines for extractable barium were calculated.

2. METHODOLOGY

2.1 Extractable Barium Regression

Soil sample concentration series from either the beginning or end of five different toxicity tests from the Stantec (2003) program were submitted to Enviro Test Labs in Saskatoon for extractable barium analysis, according to the protocol described in Section 6.2.2 in the main text. The spreadsheet received from the lab summarizing the results is included at the end of this Appendix. Analytical results are compiled in Table V-1 with an explanation of which barium acetate concentration in which toxicity test each sample came from.

Figure V-1 shows the measured extractable barium plotted against the nominal concentration of barium acetate for each of the five soil sample series. As can be seen, in Figure V-1, the five curves essentially form part of the same data set, and so it appears that the measured concentration of extractable barium is independent of the species that was present in the toxicity test, and whether the sample was collected at the beginning or the end of the test.

Figure V-2 shows the regression of the combined data set from all five soil sample series. The regression equation was $y = 0.439x - 25.180$, where x is the nominal concentration of barium acetate, and y is the measured concentration of extractable barium.

2.2 Soil Quality Guideline Calculation

Stantec (2003) (Appendix IV) provided all their toxicity data on a nominal barium acetate basis. In the current document, the 20th percentile effect data (*i.e.,* IC_{20}, EC_{20}, and LC_{20}) were used to compute the soil contact guidelines, based on guidance in CCME (2000). The nominal 20th percentile data from Stantec (2003) are summarized in Table V-2.

Table V-3 shows the results of applying the regression equation from Section 2.1 above to the nominal toxicity data, and summarizes the 20th percentile toxicity data expressed on an extractable barium basis. The different growth endpoints for each plant species were considered redundant, as were the different reproduction endpoints for springtails. Table V-3 combines each group of redundant endpoints as their geometric mean (based on guidance in CCME (2000), and calculates the soil contact guidelines for extractable barium. The soil contact guideline for natural areas, agricultural land, and residential/parkland land uses was calculated as the 25th percentile of the geometric means in Table V-3. The soil contact guideline for commercial and industrial land uses was calculated as the 50th percentile of the geometric means in Table V-3. Again, these procedures followed guidance in CCME (2000).

Table V-1. Summary of Extractable Barium Analyses

Stantec Sample Name	Test Species	Sample Collected at Day (d)	Barium Acetate Added (mg/kg)	Exchangeable Barium Measured (mg/kg)
				Springtail Acute Day 7
O.F. - ACUTE 2 - CONTROL	Springtail	7	0	41
O.F. - ACUTE 2 - 2000	Springtail	7	2,000	673
O.F. - ACUTE 2 - 4000	Springtail	7	4,000	1,419
O.F. - ACUTE 2 - 6000	Springtail	7	6,000	2,456
O.F. - ACUTE 2 - 8000	Springtail	7	8,000	3,461
O.F. - ACUTE 2 - 10000	Springtail	7	10,000	4,270
O.F. - ACUTE 2 - 12000	Springtail	7	12,000	5,337
				Earthworm Acute Day 0
E.A. - ACUTE 2 - CONTROL	Earthworm	0	0	239
E.A. - ACUTE 2 - 2000	Earthworm	0	2,000	691
E.A. - ACUTE 2 - 4000	Earthworm	0	4,000	1,612
E.A. - ACUTE 2 - 6000	Earthworm	0	6,000	2,558
E.A. - ACUTE 2 - 8000	Earthworm	0	8,000	3,459
E.A. - ACUTE 2 - 10000	Earthworm	0	10,000	4,452
E.A. - ACUTE 2 - 12000	Earthworm	0	12,000	5,321
				Invertebrate Reproduction Day 0
E.A./O.F. REPRODUCTION - CONTROL	Invertebrates	0	0	49
E.A./O.F. REPRODUCTION - 10	Invertebrates	0	10	50
E.A./O.F. REPRODUCTION - 30	Invertebrates	0	30	72
E.A./O.F. REPRODUCTION - 60	Invertebrates	0	60	68
E.A./O.F. REPRODUCTION - 100	Invertebrates	0	100	75
E.A./O.F. REPRODUCTION - 300	Invertebrates	0	300	114
E.A./O.F. REPRODUCTION - 600	Invertebrates	0	600	207
E.A./O.F. REPRODUCTION - 1000	Invertebrates	0	1,000	362
E.A./O.F. REPRODUCTION - 3000	Invertebrates	0	3,000	1,203
E.A./O.F. REPRODUCTION - 6000	Invertebrates	0	6,000	2,513
E.A./O.F. REPRODUCTION - 8000	Invertebrates	0	8,000	3,492
E.A./O.F. REPRODUCTION - 10000	Invertebrates	0	10,000	4,450
E.A./O.F. REPRODUCTION - 12000	Invertebrates	0	12,000	5,417
				Alsike Clover Definitive Day 0
ALSIKE CLOVER - DEF - CONTROL	Alsike Clover	0	0	48
ALSIKE CLOVER - DEF - 75	Alsike Clover	0	75	61
ALSIKE CLOVER - DEF - 150	Alsike Clover	0	150	75
ALSIKE CLOVER - DEF - 300	Alsike Clover	0	300	124
ALSIKE CLOVER - DEF - 500	Alsike Clover	0	500	186
ALSIKE CLOVER - DEF - 1000	Alsike Clover	0	1,000	365
ALSIKE CLOVER - DEF - 2000	Alsike Clover	0	2,000	833
				Orchard Grass Definitive Day 14
ORCHARD GRASS - DEF - CONTROL	Orchard Grass	14	0	85
ORCHARD GRASS - DEF - 75	Orchard Grass	14	75	62
ORCHARD GRASS - DEF - 150	Orchard Grass	14	150	80
ORCHARD GRASS - DEF - 300	Orchard Grass	14	300	133
ORCHARD GRASS - DEF - 500	Orchard Grass	14	500	177
ORCHARD GRASS - DEF - 1000	Orchard Grass	14	1,000	348
ORCHARD GRASS - DEF - 2000	Orchard Grass	14	2,000	744

Table V-2. Summary of Extractable Barium Analyses Based on Nominal Barium Acetate Concentrations

Effect Measurement	Endpoint	Unit	Alsike Clover	Orchard Grass	Perennial Ryegrass	Springtail Survival	Springtail Reproduction	Earthworm
Test Duration		days	21	14	14	35	35	14
Shoot length	IC20	mg/kg	859	1,105	223	-	-	-
Shoot wet mass	IC20	mg/kg	364	478	181	-	-	-
Shoot dry mass	IC20	mg/kg	294	NA	247	-	-	-
Root length	IC20	mg/kg	653	480	650	-	-	-
Root wet mass	IC20	mg/kg	351	1,000	623	-	-	-
Root dry mass	IC20	mg/kg	475	NA	556	-	-	-
Nodules	IC20	mg/kg	NA	-	-	-	-	-
adult survival	LC20	mg/kg	-	-	-	5,996	-	6000[a]
No Juveniles	EC20	mg/kg	-	-	-	-	1,158	-
Adult Fecundity	EC20	mg/kg	-	-	-	-	1,174	-

Notes:

NA = not applicable, endpoint could not be calculated

- = not measured

a LC20 not calculated - NOEC used instead

Table V-3. Summary of Extractable Barium Analyses and Soil Contact Guideline Calculation

Data Were Recalculated Using the Following Regression: **y = 0.439x - 25.180**

Effect Measurement	**Endpoint**	**Unit**	**Alsike Clover**	**Orchard Grass**	**Perennial Ryegrass**	**Springtail Survival**	**Springtail Reproduction**	**Earthworm**
Test Duration		**(days)**	**21**	**14**	**14**	**35**	**35**	**14**
Shoot length	IC20	(mg/kg)	402	510	123	-	-	-
Shoot wet mass	IC20	(mg/kg)	185	235	104	-	-	-
Shoot dry mass	IC20	(mg/kg)	154	NA	134	-	-	-
Root length	IC20	(mg/kg)	312	236	311	-	-	-
Root wet mass	IC20	(mg/kg)	179	464	299	-	-	-
Root dry mass	IC20	(mg/kg)	234	NA	269	-	-	-
Nodules	IC20	(mg/kg)	NA	-	-	-	-	-
adult survival	LC20	(mg/kg)	-	-	-	2,657	-	2,659
No Juveniles	EC20	(mg/kg)	-	-	-	-	534	-
Adult Fecundity	EC20	(mg/kg)	-	-	-	-	540	-
Geometric Mean of Redundant Endpoints		**(mg/kg)**	**230**	**339**	**187**	**2,657**	**537**	**2,659**

Natural Area, Agricultural and Residential/Parkland Guideline (25th Percentile of Geometric Means):	**257**
Commercial and Industrial Guideline (50th Percentile of Geometric Means):	**438**

Notes:

NA = not applicable, endpoint could not be calculated

- = not measured

Figure V-1. Extractable Barium Correlations

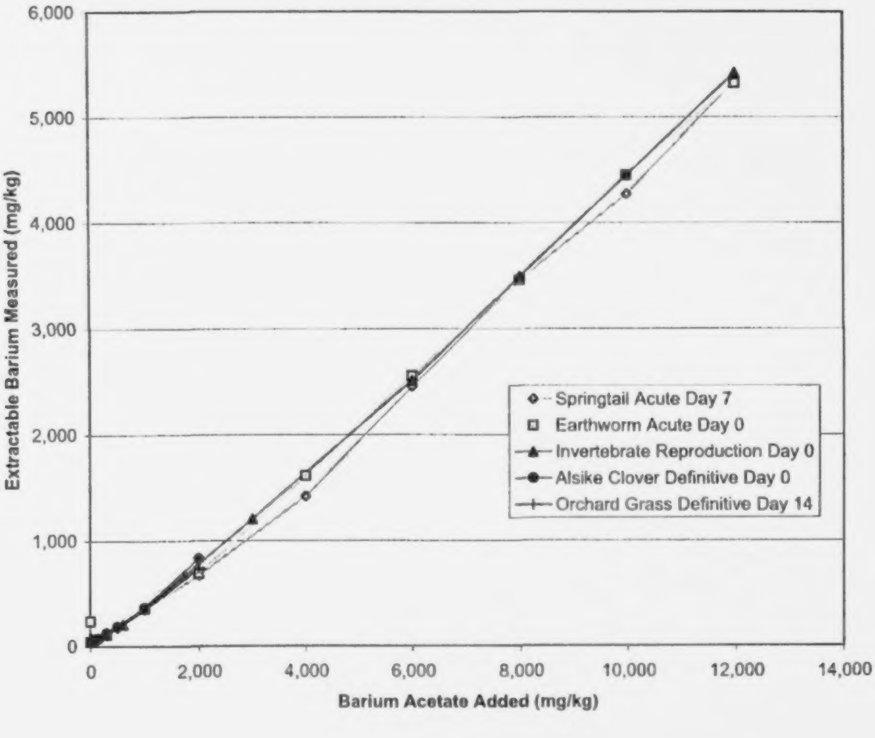
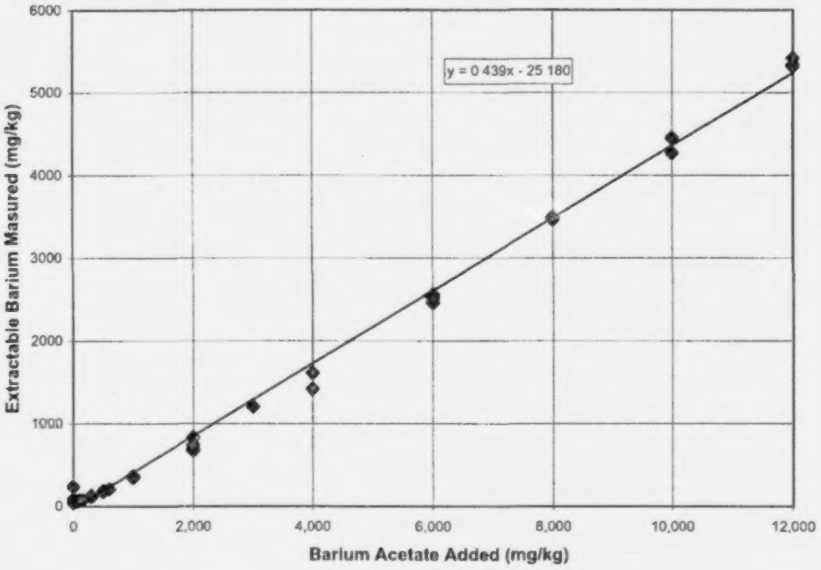


Figure V-2. Extractable Barium Regression Using All Available Data



APPENDIX VI

RUGGEDNESS TESTING FOR EXTRACTABLE BARIUM ANALYSIS

APPENDIX VI – RUGGEDNESS TESTING FOR EXTRACTABLE BARIUM ANALYSIS

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1. RUGGEDNESS TESTING

This Appendix presents the results of ruggedness testing of the soil-extraction and analysis procedure for determining extractable barium. The soil used was tested with and without a spike of barium acetate added at the rate of 160 mg Ba/kg. The coefficient of variation in the data was small, 6 - 9 %. The data indicate that there were no large or consistent effects when the following factors in the procedure were systematically changed, following Youden & Steiner (1975):

- Shaking method (platform or end-over-end).
- Shaking time (2h or 16 h)
- Filtration procedure (suction or gravity).
- Delay before extract analysis (<2 h or overnight refrigeration).
- Matrix for barium standards (water or dilute Ca chloride).
- Model of ICP spectrometer (Optima or Accuris).

The ruggedness testing included eight measurements on unspiked and spiked soil. The measurements made on each soil are summarized in Table VI-1. Results of the ruggedness testing for unspiked and spiked soil are provided in Tables VI-2 and VI-3, respectively.

The extraction ratio was always 10:1. When using 10 g of soil and 100 mL of solution, slightly more barium was extracted than with only 5 g and 50 mL. The difference was too small to justify using still larger quantities, but consistent enough to justify using no less than 10 g of soil. Recovery of the 160 mg/kg spike of soluble barium in the ruggedness tests was about 40 %. This value is slightly lower than in range-finding work using the same materials, done 2 months earlier. Available barium levels, in both the control and the spiked soil (L123906), apparently fell during storage. These effects are considered unlikely to occur with soils whose main source of barium is barite.

Overall, the ruggedness testing indicated that the method is suitably rugged for the purpose required.

2. RECOMMENDED ANALYTICAL METHOD

The recommended procedure for determination of extractable barium is as follows:

- Prepare 0.1 M calcium chloride (14.7 g of pure dihydrate per litre of de-ionized water).
- Use a representative sub-sample of air-dried, <2 mm soil, weighing at least 10 g.
- Add 0.1 M calcium chloride at a 1:10 ratio, *e.g.*, 10 g soil plus 100 mL, leaving adequate head-space for agitation.
- Agitate for at least 2 h on either a platform or end-over-end shaker.
- Filter the extract either by gravity or under suction, using a filter paper fine enough to yield a clear filtrate.
- Make up standard solutions (*e.g.* of barium acetate or chloride) either in DI water or in the extracting solution.
- Analyze the filtrate and standards by ICP within 24 h.

Table VI-1. Description of Measurements for Ruggedness Testing

#	*Extraction Quantities*	*Shaking Method*	*Shaking Time*	*Filtering Method*	*Delay in Extraction Analysis*	*Instrument Calibration Standards*	*Analytical Instrument*
1	10 g soil/100 mL 0.1 N $CaCl_2$	end-over-end shaker	overnight	suction	Next day	In $CaCl_2$	ICP-Optima
2	10 g soil/100 mL 0.1 N $CaCl_2$	end-over-end shaker	2 h	suction	<2 hours	In DI water	ICP-Accuris
3	10 g soil/100 mL 0 1 N $CaCl_2$	Platform shaker	overnight	gravity	Next day	In DI water	ICP-Accuris
4	10 g soil/100 mL 0.1 N $CaCl_2$	Platform shaker	2 h	gravity	<2 hours	In $CaCl_2$	ICP-Optima
5	5 g soil/50 mL 0 1 N $CaCl_2$	Platform shaker	2 h	gravity	<2 hours	In $CaCl_2$	ICP-Accuris
6	5 g soil/50 mL 0.1 N $CaCl_2$	end-over-end shaker	2 h	gravity	Next day	In DI water	ICP-Optima
7	5 g soil/50 mL 0.1 N $CaCl_2$	Platform shaker	overnight	suction	<2 hours	In DI water	ICP-Optima
8	5 g soil/50 mL 0.1 N $CaCl_2$	Platform shaker	2 h	suction	Next day	In $CaCl_2$	ICP-Accuris

Table VI-2. Ruggedness Testing Results for Unspiked Soil Sample

Results for Sample L123906-1

Measurement #	Ba in extract (mg/L)		Available Ba in soil (mg/kg)
1	4.07		40.7
2	4.01		40.1
3	4.28		42.8
4	4.09		40.9
5	4.31		43.1
6	3.84		38.4
7	3.74		37.4
8	3.37		33.7
mean	3.96	mean Ba (mg/kg)	39.6

Calculations				Approx % CV	9.1	%
	Factor	Difference	Abs Difference	Conf. Lts., mean +/-	7.2	mg/kg
A	Extraction Ratio	0.298	0.30	std dev.	3.6	
B	Shaking Method	0.188	0.19			
C	Shaking Time (hr)	0.273	0.27			
D	Filtering Method	-0.333	0.33			
E	Delay in Extract Analysis	-0.147	0.15			
F	Instrument calibration Standards	-0.007	0.01			
G	Instrument of Analysis	-0.057	0.06			

Ranking of Factors (most to least significant)

Factor	Abs Difference
Filtering Method	0.33
Extraction Ratio	0.30
Shaking Time (hr)	0.27
Shaking Method	0.19
Delay in Extract Analysis	0.15
Instrument of Analysis	0.06
Instrument calibration Standards	0.01

Overall Ruggedness

Standard Deviation	0.12
99% Confidence Interval (3*SD)	0.37

Table VI-3. Ruggedness Testing Results for Spiked Soil Sample

Results for Sample L123906-6

Measurement #	Ba in extract (mg/L)		Available Ba in soil mg/kg
1	10.54		105.4
2	10.01		100.1
3	11.06		110.6
4	10.09		100.9
5	10.63		106.3
6	9.88		98.8
7	9.35		93.5
8	10.43		104.3
mean	10.25	mean Ba (mg/kg)	102.5

Calculations

	Factor	Difference	Abs Difference
A	Extraction Ratio	0.353	0.35
B	Shaking Method	0.033	0.03
C	Shaking Time (hr)	0.293	0.29
D	Filtering Method	-0.333	0.33
E	Delay in Extract Analysis	0.458	0.46
F	Instrument calibration Standards	0.347	0.35
G	Instrument of Analysis	-0.568	0.57

Approx. % CV	6.4	%
Conf Lts. mean +/-	13.2	mg/kg
std dev.	6.6	

Ranking of Factors (most to least significant)

Factor	Abs Difference
Instrument of Analysis	0.57
Delay in Extract Analysis	0.46
Extraction Ratio	0.35
Instrument calibration Standards	0.35
Filtering Method	0.33
Shaking Time (hr)	0.29
Shaking Method	0.03

Overall Ruggedness

Standard Deviation	0.16
99% Confidence Interval (3*SD)	0.49